Transmission of HIV-1 Drug Resistance and Efficacy of Antiretroviral Treatment in the Swiss HIV Cohort Study

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Summary

Human immunodeficiency virus type 1 (HIV-1) has been the cause of a pandemic for more than 30 years. Without proper treatment most HIV-1 infected individuals will unavoidably progress to a condition called acquired immunodeficiency syndrome (AIDS) and eventually die. At the end of 2013, globally still more than 30 millions of people are living with HIV and more than one million people have died of AIDS (WHO 2014).
Fortunately, although HIV-1 infection currently cannot be cured, proper and continuous treatment with antiretroviral drugs can fully suppress viral replication and prevent disease progression. Standard treatment is a combination of antiretroviral treatments (cART), mostly consisting of two nucleos(t)ides analogue inhibitors (NRTIs) and one potent agent from either the protease inhibitors (PIs), the non-nucleotide analogue inhibitors (NNRTIs), or other new drug classes. Modern drugs are more potent and less toxic and thus are more appropriate for long-term usage. However, due to the virus's ability to mutate rapidly and to generate large genetic diversity, drug-resistant mutants can emerge.
Drug resistance is a major concern jeopardizing the treatment success. There are two types of drug resistance: acquired and transmitted. Whereas the former is mostly selected, e.g., due to suboptimal treatment or insufficient adherence, the latter is transmitted from either a treatment-failing or treatment-naïve individual who carries the drug-resistant virus. In most industrialized countries acquired drug resistance has been reduced substantially in terms of prevalence thanks to successful treatment; however, on the contrary, prevalence of transmitted drug resistance has not declined. This is contradictory to the general hypothesis that transmission of drug resistance is primarily driven by patients with acquired drug resistance. In Research Project 1 we analyzed the resistance and clinical data from 2421 recently-infected, treatment-naïve and 5399 treatment-failing patients and throughly studied prevalence of transmitted drug resistance over 15 years in Switzerland, including its time trend and associated risk factors and its association with the viral burden of treatment-failing patients. We found that prevalence of transmitted drug resistance fluctuated considerably over time; it dropped sharply when a new drug class was introduced but increased in the years when no new drug class was introduced. Therefore, introduction of new drug classes kept transmission of drug resistance low over time and will most likely be needed in the future to maintain low drug resistance transmission rates. Moreover, we found that treatment-naïve patients also represent a major transmission reservoir for drug resistant viruses, mostly harboring low-fitness-cost mutations.
In Research Project 2 we determined the persistence behavior of 17 transmitted drug resistance mutations by calculating reversion rates in treatment-naïve patients, and estimated the association of the persistence with the predicted fitness cost of individual mutations in the genetic backgrounds in which they occurred using a previously published machine-learning algorithm. We could show strong variations in the persistence behavior of transmitted drug resistance mutations and the significant association of persistence with predicted fitness costs. Specifically, we found that even mutations of the same type tended to persist longer if they occurred in a genetic background where they caused weak fitness costs.
In Research Project 3 we moved from transmitted to acquired drug resistance. Over time, the most recommended and used NRTI backbones for firstline ART have changed. From the oldest combination zidovudine(AZT)/lamivudine(3TC), to the tenofovir(TDF)/3TC, to recently abacavir(ABC)/3TC and TDF/emtricitabine(FTC). The daily dosing frequency and the daily number of pills that need to be taken (pill burden) vary substantially among these four combinations, which could be an important confounder when studying the relative efficacy. We thus compared the relative treatment efficacy rearding viral re-

sponse and the emergence of NRTI resistance and adjusted the model for dosing frequency and pill burden. Our results showed robust associations of pill burden and ethnicity with treatment efficacy.
In conjunction, we performed in depth analyses of the epidemiology of transmitted drug resistance over time, studied the association of fitness costs with differential persistence behavior of transmitted drug resistance mutations, and compared the risk of having virological failure and emergence of acquired drug resistance among different NRTI backbones.

Zusammenfassung

Das Humane Immundefizienz-Viurs (HIV) ist die Ursache einer globalen Epidemie seit rund 30 Jahren. Eine HIV-Infektion wird sich, ohne richtige Behandlung, zu AIDS entwickeln, und die Infizierten werden mit wenigen Ausnahmen schliesslich daran sterben. Ende 2013 leben über 30 Millionen Menschen mit HIV-Infektion und über eine Millionen Menschen starben an AIDS (WHO 2014).
Eine HIV-Infektion kann nicht ganz geheilt werden, allerdings kann mit angemessenen und kontinuierlichen Therapien die Vermehrung der HI-Virus unterdrückt werden, und dadurch die Progression der Infektion unterbinden. Eine Standardtherapie besteht in der Regel aus drei Medikamenten, am häufigsten jeweils zwei nukleosidische Reverse-Transkriptase-Inhibitoren (NRTI) plus einen nicht-nukleosidische-Reverse-Transkriptase-Inhibitor (NNRTI) oder einen Proteaseinhibitor (PI). Modernere Therapien sind im Vergleich mit älteren Medikamenten potenter und haben weniger Lanzeittoxizität, und sind somit besser geeignet für langjährige Behandlungen. Dennoch können gelegentlich Resistenzen gegen diese Medikamente entstehen.
Die Resistenzen sind problematisch, denn sie gefährden den Erfolg der HIV-Therapien. Es gibt zwei Arten von Resistenzen: erworbene und übertragene Resistenz. Die Erstere wird durch antiretrovirale Medikamente selektioniert, wohingegen die Letztere von einer Person übertragen wird, die resistente Viren in sich trägt. In den meisten industrialisierten Ländern konnte die Prävalenz der erworbenen Resistenz in den letzten Jahren durch erfolgreiche Therapien reduziert werden. Dies ist allerdings nicht der Fall für übertragene Resistenz. Dieses Phänomen widerspricht der allgemeinen Hypothese, dass Resistenz primär von den Therapie-Versagern übertragen wird. Im Forschungsprojekt 1 haben wir die Resistenzdaten und klinische Daten von 2421 neu infizierten und unbehandelten Patienten und 5399 Patienten, die eine fehlgeschlagene Therapie erlitten haben, analysiert. Wir haben die Prävalenz der übertragenen HIV-Resistenz über den Zeitraum von 15 Jahren in der Schweizerischen HIV-Kohortenstudie untersucht, inklusive assoziierter Risikofaktoren, und die Assoziation mit der Viruslast von Therapie-Versagern, studiert. Wir haben festgestellt, dass die Prävalenz der übertragenen Resistenz stark geschwankt hat. Sie ist jeweils angestiegen in der Zeit, während der keine neue Medikamentenklasse eingeführt wurde, und hat andererseits jeweils abgenommen, wenn neue Medikamentenklassen eingesetzt werden konnten. Diese Resultate deuten darauf hin, dass einerseits die Einführung einer neuen Medikamentenklasse die Übertragungsrate der Resistenz zwar für eine gewisse Zeit reduziert, anderseits bedarf es ständig neuer Medikamente, um die Übertragungsrate tief zu halten. Wir haben zusätzlich herausgefunden, dass unbehandelte Patienten ein wichtiges Reservoir für übertragene Resistenzen bilden, vor allem für solche, die tiefere Fitnesskosten haben.
Im Forschungsprojekt 2 haben wir mit *in vivo* Daten von unbehandelten Patienten die Persistenz von 17 übertragenen Resistenzmutationen bestimmt, indem wir die Reversionsraten kalkuliert haben. Ein machine-learning Algorithmus wurde benutzt, um die Fitnesskosten individueller Mutationen in dem genetischen Hintergrund, in dem eine Mutation auftritt, vorauszuberechnen. Wir haben gezeigt, dass die Persistenz einer Mutation stark variiert und mit den Fitnesskosten assoziiert ist. Wir haben auch gezeigt, dass sogar der gleiche Mutationstyp eine längere Reversionsrate hat, wenn sie in einem genetischen Hintergrund auftritt, der tiefere Fitnesskosten verursacht.
Im Forschungsprojekt 3 haben wir uns auf erworbene Resistenz konezentriert. Im Speziellen haben wir die Wirksamkeit und die Resistenzbildung der zu unterschiedlichen Zeiten am häufigsten gebrauchten NRTI-Kombinationen, Zidovudine (AZT)/Lamivudine(3TC), Tenofovir(TDF)/3TC, Abacavir(ABC)/3TC und

TDF/Emtricitabine(FTC), in Abhängigkeit von verschiedenen Faktoren verglichen. Wir untersuchten einerseits das virologische Versagen (engl: virological failure, VF) und andererseits die Bildung von NRTI-Resistenz zwischen den vier NRTI Gruppen. Diese Kombinationen unterscheiden sich auch durch die Dosierungsfrequenz und die Tagesanzahl der einzunehmenden Pillen (engl: pill burden). Wir haben diese beiden Faktoren in die Analyse deswegen miteinbezogen. Unsere Resultate haben gezeigt, dass der "Pill-burden" und die Ethnie eine wichtige Rolle spielten für das Therapieversagen und für die Entwicklung von Resistenz.
Zusammengefasst habe wir detailliert die Epidemiologie der übertragenen Resistenzen im Verlauf der Zeit analysiert. Weiter haben wir die Assoziation der Fitnesskosten mit der verschiedenen Persistenz der Resistenzmutationen *in vivo* studiert. Zuletzt haben wir das Risiko, das zum Versagen einer Therapie und zur Bildung erworbener Resistenzmutationen führt, bei verschiedenen NRTI Kombinationen verglichen.

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Introduction

1 HIV Infection

The infection by the human immunodeficiency virus-1 (HIV-1) ultimately leads to death in almost all patients if untreated. Globally, to date almost 78 million people have been infected with HIV and of those about 39 million people have died (WHO 2014). In 2013, still more than 30 million people are living with HIV and more than 1 million have died of AIDS (WHO 2014). It was estimated that 2.7 million people have become newly infected in the year 2010; most of them live in low- and middle-income countries (UNAIDS 2013). HIV causes the acquired immunodeficiency syndrome (AIDS) [1–3]. It induces a CD4 decline over the years that results in severe immunodeficiency followed by opportunistic diseases such as bacterial, fungal and viral opportunistic infections and HIV-associated cancers [4–7]. Combination antiretroviral treatment introduced in 1996, a combination of three different antiretroviral agents, has fundamentally changed the course of disease and has turned a deadly disease into a chronic disease. With proper treatment nowadays, viral replication in most patients can be fully suppressed [8–10]. This results in a recovery of the CD4 T cells in patients who had already suffered from AIDS defining illnesses and prevents a CD4 cell decline in patients who are diagnosed during early disease stages.
HIV infects people through the transfer of body fluids, including blood, semen, pre-seminal fluid, rectal fluid, vaginal fluid, and breast milk. The major transmission routes are sexual contact (including men having sex with men and heterosexual transmission), intravenous drug abuse, and mother to child transmission. Data from the Swiss HIV Cohort Study showed that 61% of male patients were infected via homosexual contact, 24% male and 76% female patients via heterosexual contact, and 9-10% of patients in both genders via intravenous drug abuse. In the early days, HIV was also transmitted by contaminated blood products. However, HIV is not spread by air, water, saliva [11], sweat, casual body contacts like shaking hands and hugging, or contact with the same toilet seats.
Though HIV infection has been notorious for almost 30 years, its advanced treatment is proven to be able to considerably reduce mortality [5, 12, 13]

1.1 Origin and history

The HIV-1 epidemic is assumed to originate from a crossover of the simian immunodeficiency virus (SIV) from chimpanzees to humans [14–16]. In the early 1980s, AIDS was identified in the United States and started to cause global attention [17–19]. In 1983 Gallo's and Montagnier's labs isolated independently for the first time the virus causing AIDS from AIDS patients [20, 21]. In 1986 this virus was named HIV.

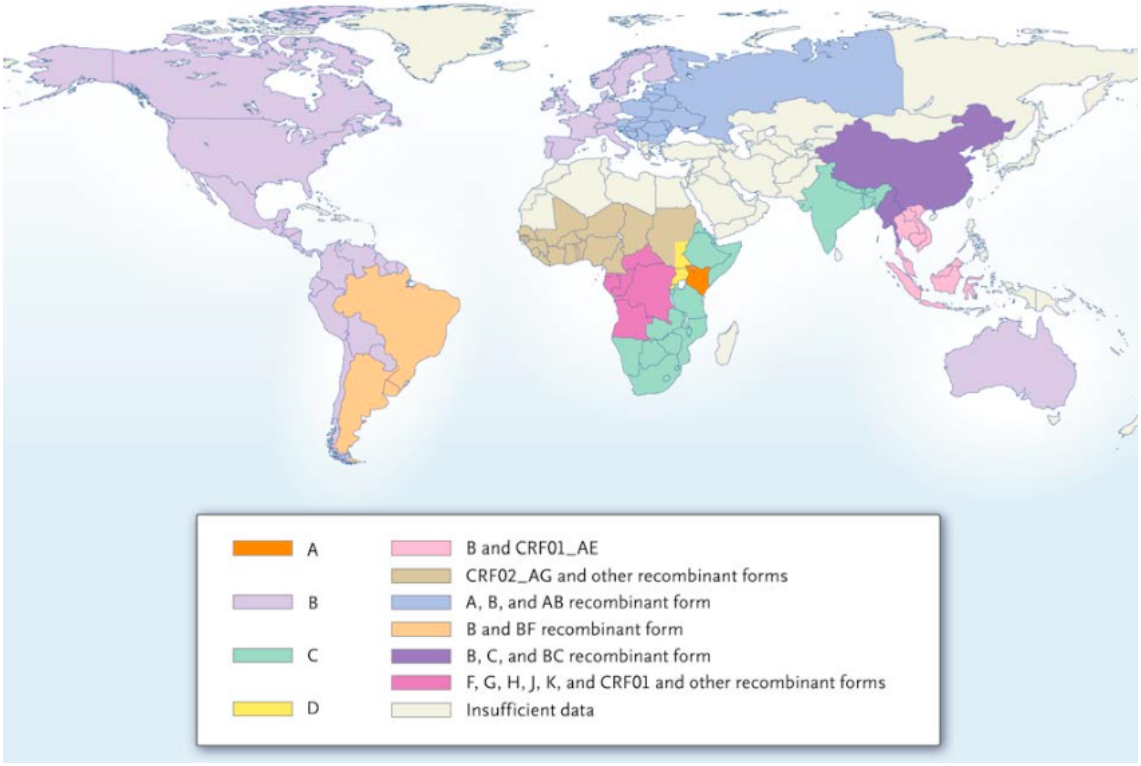

Figure 1.1: Global distribution of HIV-1 subtypes and circulating recombinant forms [34]

1.2 Types and subtypes

There are two types of HIV, type 1 and type 2. While HIV-1 and HIV-2 can both cause AIDS, the former was found to be much more prevalent [22], virulent and infectious [23]. Due to the low infectivity HIV-2 remains mostly restricted to West Africa [24]. The notorious pandemic HIV infection is actually caused by HIV-1, and in particular, the group M.

HIV-1 is divided into four groups based on genetic differences: M, N, O, and P [25]. Infections with HIV-1 other than from the group M are extremely rare, and are only found in individuals from Cameroon [25]. Group M is further divided into nine subtypes as well as many circulating recombinant forms among subtypes due to recombination [16]. There is no conclusive evidence that one subtype is more infectious than the others. All subtypes can be reliably treated with current antiretroviral agents [26–28].

The distribution of HIV subtypes and circulating recombinant forms is illustrated in Figure 1.1. Worldwide $\approx 50\%$ of the HIV-1 infected population harbors subtype C viruses making subtype C the most dominant subtype, followed by subtype A ($\approx 12\%$) and subtype B ($\approx 10\%$) [16, 29]. However, subtype C is mostly concentrated in southern Africa and India. Subtype A and A recombinant forms are predominant in central and eastern Africa and in eastern Europe [16]. The largest diversity can also be found in central Africa where all subtypes and many recombinants are present [29, 30]. On the contrary, subtype B is the most widely spread and well studied subtype because it is the dominant subtype in western and central Europe, the Americas, and Australia [16, 30]. However, with increasing tourism and networks between these countries and low- and middle-income countries, an increasing prevalence of non-B subtypes has appeared in many high-income countries [31–33].

1.3 The natural course of HIV-1 infection

There are several different phases from a new HIV-1 infection progressing to AIDS, each being characterized with distinct disease attributes. Although the time course of an infection can vary substantially from person to person, the general progression steps are fundamentally similar (Figure 1.2).
The HIV-1 infection usually starts with an asymptomatic phase (referred to as the eclipse phase) that lasts one to two weeks. The virus can replicate and migrate in the blood freely because the immune response is not active yet. During this phase, viral RNA in the plasma cannot be detected by any clinical diagnostic assay [35].
The following weeks are characterized by a short drop of CD4 counts and a sudden increase of viremia. This phase is referred to as the primary or acute infection phase. About 60-80% of cases present unspecific symptoms resembling a "viral syndrome", e.g., acute mononucleosis including fever, headaches, sore throat, skin rash, lymphadenopathy, diarrhea, etc [36, 37]. The immune response begins to be active at the time of the viremia peak, leading to the production of a large number of activated CD4 cells. As a result of both partial control of the immune system and exhaustion of activated CD4 cells, high viremia decreases strongly at the end of this phase ($\approx$ 100 days following the infection) and begins to plateau [38]. During this phase, there is generally a sequential gain in positive clinical diagnostic assays, starting from viral RNA measured by PCR, to p24 viral antigen measured by enzyme-linked immunosorbent assay (ELISA), to HIV-1 specific antibody detected by ELISA, and finally to HIV-1 specific antibody detected by ELISA and western blot [35].
The next phase following the primary infection can last up to decades and is thus called the phase of clinical latency. This phase is usually asymptomatic. Over time, viremia starts to increase steadily. Everyday there is a large number of activated CD4 cells becoming infected and dying. Most infected individuals will gradually progress to the phase of AIDS (median time: 8-10 years) with the exception of elite controllers. Elite controllers are a rare group of individuals who are able to maintain a high number of activated CD4 cells and a low level of viremia. Unfortunately, it is not known exactly how these people can control the infection better than others without progressing to the final phase, AIDS [1–3, 39–41].
When CD4 cell counts drop to below $\approx 200/\mu\ell$, severe immunodeficiency occurs. AIDS defining illnesses and occasionally other opportunistic infections will start to show [42–44]. Control of HIV through the immune system is almost completely lost causing viremia to start to increase rapidly. Eventually, an untreated patient will inevitably die.

1.4 Disease progression and monitoring

In the initial phase of the HIV-1 infection the viral population in most cases (60-90% in sexual transmission) is monomorphic [45, 46]. With time genetic diversity gradually accumulates until a plateau is reached [47, 48]. Therefore, genetic diversity can be used as an indicator to determine the age of the infection (more on genetic variability see Chapter 4.1). The ambiguity score, which was developed in our group by Kouyos et al., is a simple measure for viral diversity based on ambiguous nucleotide calls from routine population sequencing as performed for genotypic resistance testing [49]. The infection age was found to be correlated with the ambiguity score. It is a useful tool to distinguish between recent and chronic infection in patients lacking seroconversion data, which is often the case in large cohort studies. This difficult to answer but highly relevant question can now be addressed more appropriately without further costs, in particular for research

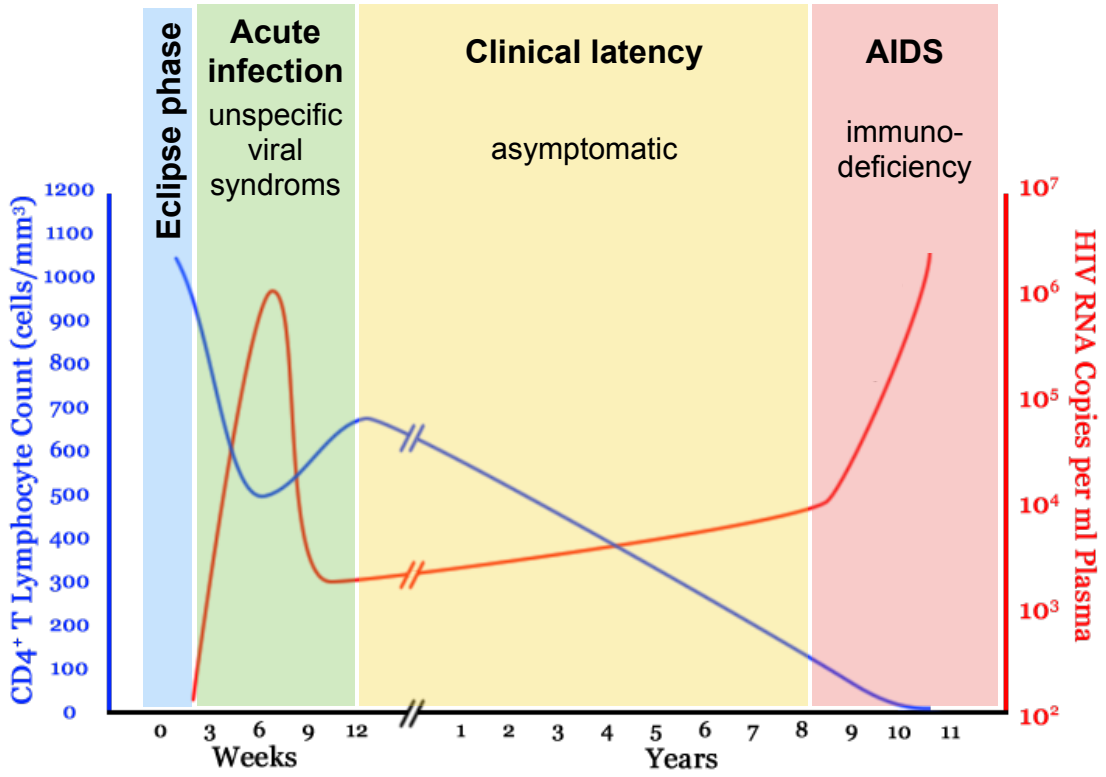

Figure 1.2: The natural time course of HIV infection

(adaped from [38])

purposes [50–52].
The two major surrogate markers for disease progression are the CD4 cell count and the viral load (HIV-plasma RNA). The latter is also the key parameter to monitor treatment success [53] and adherence to therapy (for treatment recommendations see Chapter 3.4).

2 HIV-1 Pathogenesis

2.1 Virus structure

HIV belongs to the group of retroviruses and possesses a genome of single-stranded RNA, which is 9749 bp long. The virus comprises two copies of single stranded RNA enclosed by three layers of protection. From the innermost to the outermost layer, we have a conical capsid composed of the viral protein p24, a matrix composed of the viral protein p17, and finally an envelope composed of some of the host cell membrane and glyocoproteins including gp120 and gp41 [54]. Together with the viral RNAs, accessory gene products such as Vif, Nef, Vpr and essential viral proteins are enclosed within the capsid. Gp120 is anchored to the viral membrane, or envelope, via non-covalent bonds with the transmembrane glycoprotein, gp41. Three gp120s and gp41s form a trimer of heterodimers [55, 56]. Approximately 6-10 of these trimers sit on a virion and form the envelope spikes that are essential for entering new host cells [57, 58] (Figure 2.1).
HIV genome comprises three major genes, encoding functional and structural proteins, and other regulatory genes (Figure 2.2). The *gag* gene encodes the capsid and matrix materials, the *pol* gene is responsible for essential viral enzymes including reverse transcriptase, protease and integrase, and the *env* gene produces envelope-associated proteins, gp120 and gp41.

2.2 Life cycle

The life cycle of HIV-1 is composed of several steps:

1. viral entry into the host cell
2. reverse transcription of the viral genome
3. integration of the viral DNA
4. assembly, release, and maturation

For entry, HIV needs both the CD4 glycoprotein and a chemokine receptor on the surface of the target cell including CD4 cells, macrophages, and dendritic cells. After gp120 successfully binds to the receptor CD4 molecule on the target cell surface [59, 60], gp41 undergoes a significant conformational change allowing the co-receptor binding, which is mediated partially by the V3 loop attached to the gp120 [61, 62]. Following the co-receptor binding gp41 continues to fold into six helix bundles bringing the viral membrane and the

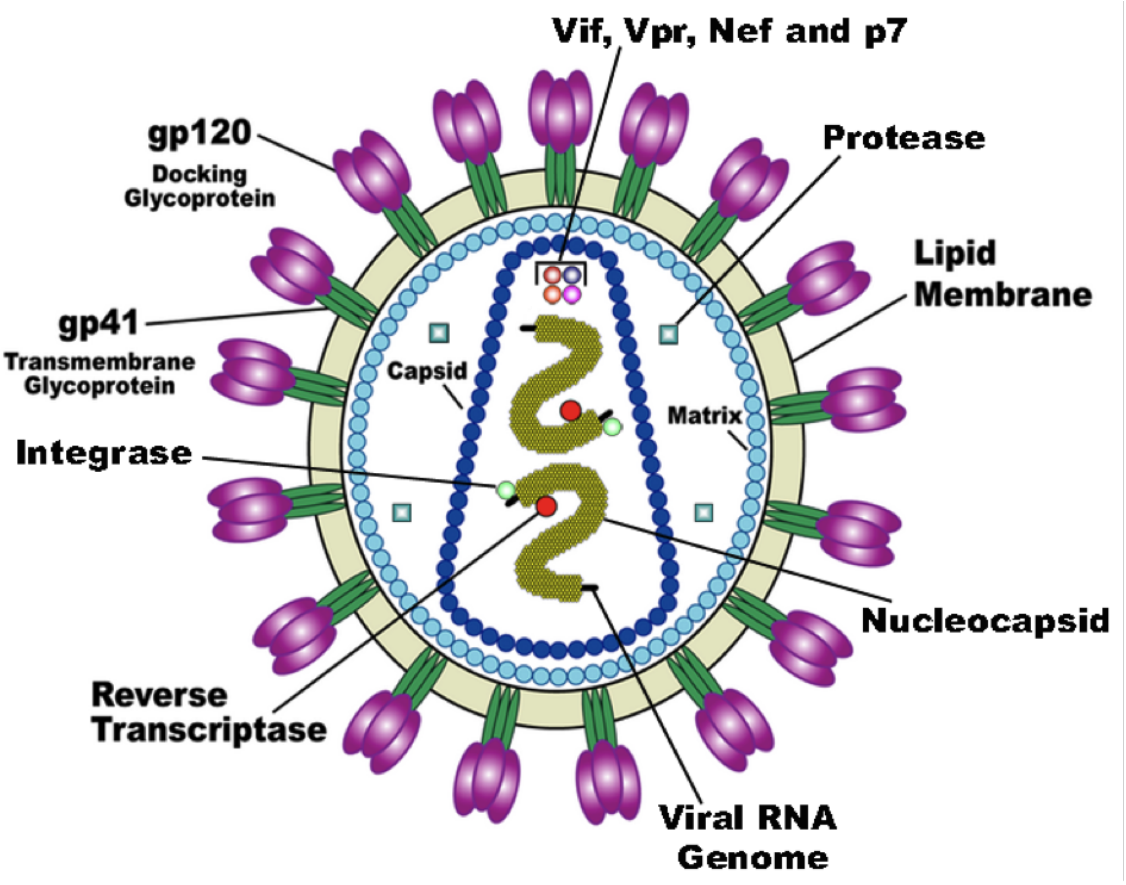

Figure 2.1: HIV structure

(from U.S. department of health and human services: `http://web.archive.org/web/20050531012945/http://www.niaid.nih.gov/factsheets/howhiv.htm`)

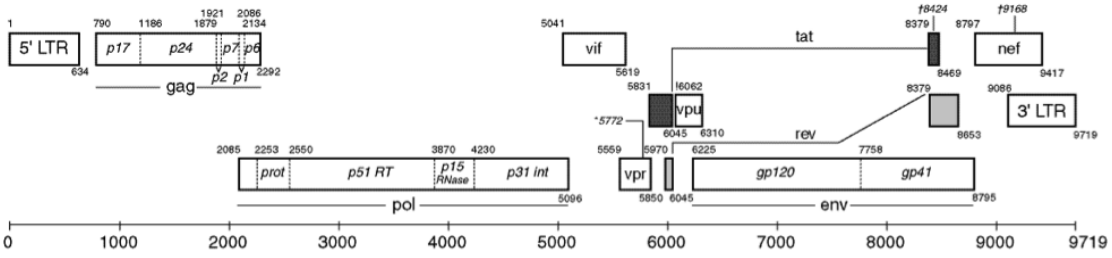

Figure 2.2: HIV-1 gene map

(from `http://www.hiv.lanl.gov/content/sequence/HIV/MAP/landmark.html`)

host cell membrane closer [63, 64] and leading finally to the fusion of the two membranes. An entry pore is created that allows the viral capsid to enter the target cell [65].
There are two kinds of chemokine receptors that HIV uses as the co-receptor for entry, CCR5 and CXCR4 – this phenomenon is called viral tropism. Viruses that use CCR5 are called R5 tropic, viruses that use CXCR4 are called X4 tropic, and viruses that are able to use both CCR5 and CXCR4 are called R5X4 dualtropic [66–68]. With rare exceptions, most viruses being transmitted use CCR5, either completely or partially [45, 46]. Viruses using CXCR4 emerge later in the course of the infection with a percentage of around 50% [69–71].
After entry, viral enzymes along with the viral genome are released into the host cell. Reverse transcriptase transcribes single-stranded RNA into single-stranded cDNA, and subsequently synthesizes a sense DNA for the antisense cDNA creating a double-stranded DNA. This DNA is then transported into the cell nucleus and integrated into the host genome by the viral enzyme integrase [72, 73]. The integrated viral DNA is called the provirus. The next steps are executed by taking advantage of functions of human RNA polymerase and tRNA: First, the HIV provirus is transcribed into mRNA, which is spliced into smaller pieces so that the smaller RNA pieces can be transported from the nucleus to the cytoplasm. In the cytoplasm, viral proteins are translated via human tRNAs. The viral regulatory protein Rev is one of the viral proteins that are translated the earliest because this protein carries out a very important function, namely exporting the unspliced viral mRNA from the nucleus to the cytoplasma [74, 75]. Once the full-length mRNA reaches the cytoplasm, large proteins like Gag and Gag-Pol proteins can then be translated. Finally, the full-length RNA together with Gag and Gag-Pol proteins and viral protease bud through the host cell membrane [76].
Accompanying (or immediately following) budding, the final step of the life cycle begins [77]. Protease cleaves large viral protein precursors into structural proteins and functional enzymes. Only after the maturation process, the virus is infectious [78–80].

2.3 Latent HIV Reservoir

The HIV-1 latent reservoir is formed by replication-competent viruses accumulated primarily in resting memory CD4 T cells. Due to the long lifespan of resting CD4 cells, these viruses are more stable than the actively replicating viruses, and show only minimal decay even under successful antiretroviral treatment [81]. Thus, even when viremia levels are suppressed below the detection limit, the latent reservoir is sufficient for a lifelong persisting infection [82, 83]. With current treatment options, eradication of the latent reservoir cannot be achieved [83–85].
Latency is established at very early stage after infection [86, 87], when an active CD4 cell becomes infected and survives long enough to revert back to the resting state [88]. However, following infection of the resting CD4 cells, sometimes a block occurs probably at the stage of importing the viral DNA into the nucleus. This results in the preintegration latency [89]. Whereas the preintegration latency is characterized with unintegrated HIV-1 DNA and is of little clinical relevance due to its labile nature, the postintegration latency establishes a stable latent reservoir with integrated viral DNA, which is reversibly silenced, in the host genome [90, 91].
Evidence was found to support that transcription of unspliced RNA can occur constantly. This phenomenon is partially responsible for the residual viremia (average level of 1 copy/$m\ell$) [88, 92, 93], which cannot be reduced by treatment intensification [94–96]. Moreover, during active viral replication, new variants that evolve over time also continuously enter the latent reservoir [97]. This means that in treatment-failing patients with drug re-

sistance, resistant viruses are deposited in the latent reservoir and can potentially reemerge if an antiretroviral drug that viruses are resistant to is used.
In conclusion, latency is responsible for the need for lifelong treatment of HIV-1 infected individuals (i.e., there is viral rebound after therapy discontinuation [98]) and persistence of drug resistance (also see Chapter 3 and Chapter 4 for antiretroviral treatment and drug resistance, respectively).

3 HIV Antiretroviral Treatment

HIV-1 infection is a lifelong infection for which there is currently no cure. Today there are more than 25 antiretroviral agents from 5 different drug classes available to treat HIV-1 infected patients. Although it is not possible to eliminate HIV-1 infection, potent combination of antiretroviral treatment (cART) can fully inhibit viral replication and stop viral evolution [98]. Moreover, effective treatment can also reconstitute the immune system and prevent its destruction [53]. Generally, plasma HIV-1 RNA in treated patients is below the detection limit of the most sensitive assays used in clinical practice. However, if patients stop treatment, plasma HIV-1 viral load rebounds eventually within days to weeks in the majority of patients [99]. Factors leading to treatment failure (i.e., when undectable plasma HIV-1 RNA levels are not achieved or maintained) are, e.g., non-adherence, drug intolerability, drug-drug interactions with another HIV-1 antiretroviral agent or other medications that reduce optimal drug levels in the blood, transmitted drug resistance, emergence of drug resistance on treatment, dosage errors, and drugs of low potency [53].

3.1 Available drugs

The first HIV-1 antiretroviral drug, zidovudine or azidothymidine (AZT), was approved in Switzerland in 1987 [105, 106]. AZT is a nucleoside analogue reverse transcriptase inhibitor (NRTI), which blocks the reverse transcriptase's enzymatic function by terminating the DNA polymerization. Many drugs have been developed and approved over time [53]. To date, 29 approved and registered HIV antiretroviral drugs including co-formulated tablets are prescribed for HIV-1 infected patients in Switzerland. Table 3.1 summarizes all approved single-formulated HIV antiretroviral drugs in Switzerland.
In addition to these drugs, several co-formulations containing fixed doses of two or three drugs from one or two drug classes have also been developed. See Table 3.2 for the registration time line.

3.2 Mechanisms of antiretroviral agents

All HIV antiretroviral agents are categorized into classes according to their targets and functions. NRTIs are mostly nucleoside analogues with the exception of tenofovir being a nucleotide analogue. NRTIs need to be phosphorylated and are only active as triphosphates. They compete with the natural deoxynucleotides (dNTP) in hosts for incorporation into the growing viral DNA chain. However, because NRTIs lack a 3'-hydroxyl group

Generic Name	Abbreviation	Brand Name	Registered Year	Drug Class
Zidovudine	AZT	Retrovir	1987	NRTI
Didanosine	DDI	Videx	1992	NRTI
Saquinavir	SQV	Invirase	1996	PI
Lamivudine	3TC	3TC	1996	NRTI
Stavudine	d4T	Zerit	1996	NRTI
Indinavir	IDV	Crixivan	1996	PI
Ritonavir	RTV	Norvir	1996	PI
Nelfinavir	NFV	Viracept	1997	PI
Nevirapine	NVP	Viramune	1997	NNRTI
Efavirenz	EFV	Stocrin	1998	NNRTI
Amprenavir	APV	Agenerase	1999	PI
Abacavir	ABC	Ziagen	1999	NRTI
Lopinavir + Ritonavir	LPV/RTV	Kaletra	2000	PI
Didanosine	ddI	Videx Ec	2001	NRTI
Tenofovir	TDF	Viread	2002	NRTI
Enfuvirtide	T-20	Fuzeon	2003	Fusion Inhibitor
Atazanavir	ATV	Reyataz	2004	PI
Emtricitabine	FTC	Emtriva	2004	NRTI
Fosamprenavir	FOS-APV	Telzir	2005	PI
Tipranavir	TPV	Aptivus	2005	PI
Darunavir	DRV	Prezista	2006	PI
Maraviroc	MVC	Celsentri	2008	CCR5 antagonist
Raltegravir	RGV	Isentress	2008	InSTI
Etravirine	ETR	Intelence	2008	NNRTI
Rilpivirine	RPV	Edurant	2013	NNRTI
Elvlitegravir [a]	EVG	-	2013	InSTI
Dolutegravir	DTG	Tivicay	2014	InSTI

a. Only available in the single tablet regimen containing EVG, TNF, FTC

Table 3.1: Approved single-formulated HIV antiretroviral drugs

Brand Name	Containing drugs	Registered Year	Drug Classes
Combivir	AZT, 3TC	1998	NRTI
Trizivir	AZT, 3TC, ABC	2000	NRTI
Kivexa	ABC, 3TC	2005	NRTI
Truvada	TDF, FTC	2006	NRTI
Atripla	TDF, FTC, EFV	2009	NRTI, NNRTI
Eviplera	TDF, FTC, RPV	2011	NRTI, NNRTI
Stribild	TDF, FTC, EVG	2013	NRTI, InSTI
Triumeq	ABC, 3TC, DTG	2015	NRTI, InSTI

Table 3.2: Approved co-formulated HIV antiretroviral drugs

on the deoxyribose unlike the natural dNTP, the incorporation of a NRTI prevents the next incoming dNTP from forming the 5'-3' bond, which is essential for DNA synthesis. As a result, after incorporation of NRTI into the DNA chain, DNA polymerization is terminated [107–109].

Protease inhibitors (PI) are a class of drugs that bind the viral protease and prevent protease from cleaving the viral protein precursors. This class of drugs binds to the substrate/inhibitor binding site of the protease and reduces the enzyme's catalytic activity [110, 111]. The first PI, Saquinavir, was developed and introduced after NRTI in 1996, being the second class of HIV antiretroviral drugs. In 2000, a milestone was set when ritonavir-boosted PIs were introduced [112] because boosted PIs had increased efficacy, high barrier of resistance, and a reduced pill burden and dosing frequency [113–115]. Recently, a novel booster, cobicistat, was developed and demonstrated similar efficacy to RTV. Since it has no antiretroviral activity *in vitro*, it eliminates any potential for PI resistance to emerge [116, 117].

Non-nucleoside reverse transcriptase inhibitors (NNRTIs) are the third class of antiretroviral drugs that was developed. Unlike NRTIs, NNRTIs employ a different mechanism to block reverse transcriptase's enzymatic function. NNRTIs are small molecules with strong affinity for a hydrophobic pocket, which is located near the reverse transcriptase's catalytic domain. Binding of NNRTIs to the reverse transcriptase directly influence the flexibility of the enzyme so that the distorted part cannot bind to DNA, resulting in the inhibition of DNA polymerization [118, 119].

In addition to the aforementioned classes that comprise the most frequently used HIV therapies, drugs targeting other functional parts along the HIV life cycle have also been developed. One of the novel classes is the entry inhibitors, which consist of maraviroc, a CCR5 antagonist, and enfuvirtide, a fusion inhibitor. Maraviroc is the only antiretroviral drug so far that targets a host cell compartment, i.e., CCR5, instead of the virus. CCR5 is a co-receptor for most HIV strains on the surface of human macrophages and T-cells and is necessary for the virus to enter the host cell. Maraviroc binds to CCR5 and prevents CCR5 from associating with the viral envelope glycoprotein [120, 121]. However, maraviroc is not effective against HIV with X4 or R5X4 tropism. Therefore, a HIV tropism test is mandatory before a patient starts the treatment containing maraviroc. Enfuvirtide, on the other hand, works at the final stage of the entry process; it inhibits membrane fusion.

Enfuvirtide is a peptide consisting of 36 amino acids that binds to the HR2 region of gp41. The creation of an entry pore is thus prevented, keeping the capsid of the virus out of the target cell [122]. The advantage of enfuvirtide is that its target is novel, thus making it important especially as salvage treatment for patients with multiple resistances. However, as enfuvirtide needs to be injected twice a day due to a short half-life and has relatively low potency, its clinical application is very limited today [123].
Another novel class is the integrase inhibitors (Integrase Strand Transfer Inhibitor, InSTI), which prevent viral DNA from being integrated into the host DNA. The first integrase inhibitor, raltegravir, was approved by Swissmedic in 2008 first for salvage therapy and has resulted in superior viral suppression with optimized background therapy than optimized background therapy alone for at least 48 weeks [124].

3.3 Combination of antiretroviral drugs

Before the first PI or NNRTI were developed and approved, HIV-1 infected patients were treated with AZT monotherapy [106], or AZT/DDI [125], AZT/ddC or AZT/3TC [126] dual therapy. These treatments had a modest effect in decreasing mortality and could only partially achieve viral suppression. In 1996, a new form of therapy against HIV-1 infection was introduced [127, 128]. To distinguish from the early ART, this new form of therapy was termed potent combination antiretroviral therapy (cART), or later on highly active antiretroviral therapy (HAART). It combines 2 NRTIs plus a potent antiretroviral agent from another drug class, usually a PI (initially non-boosted and after 2000 mostly boosted), a NNRTI, or an integrase inhibitor (used increasingly since 2010) [53, 129, 130]. Today, it is generally believed that HAART can stop viral replication in most patients completely, although there have been ongoing discussions whether low level replication might occur in some patients or in some tissues [131]. However, the lack of evolution under HAART and the absence of increasing treatment failures and emergence of resistance at the population level so far are arguments against ongoing low level replication [98, 132–134].
HAART has remained the gold standard of anti HIV-1 treatment since introduction in 1996. It was also proven in our cohort, the Swiss HIV Cohort Study (SHCS), that cART is highly effective in achieving viral suppression and reducing HIV-related morbidity and mortality dramatically [13].

3.4 Treatment policy

There has been a constant debate since introduction of antiretroviral therapy as when to start a treatment. Since HIV-1 infection is a chronic disease and needs to be treated lifelong with currently available drugs, not only maximal viral suppression and preservation of the immune system but also the quality of a patient life is of high importance regarding the time of initiation of treatment. The optimal time to start cART depends therefore on the treatment benefits in balance with the downsides of long-term therapy such as drug toxicity and potential emergence of viral drug resistance.
Before 2008, it had been constantly recommended that all HIV-1 infected patients with HIV-related symptoms and all asymptomatic HIV-1 infected persons with CD4 cell count $\leq 200/\mu\ell$ should be treated [135]. The recommendation in 2008 for treatment initiation from the international AIDS society-USA panel lifted the treatment threshold to CD4 counts $< 350/\mu\ell$ for all asymptomatic HIV-1 infected patients [136] because studies were published supporting benefits of treating patients at CD4 cell levels of $350/\mu\ell$ or above [137–140] without increasing the risks for adverse events caused by drug toxic-

ity [141]. Moreover, newer medications are more potent and less toxic [142, 143], resulting in reduced worries of substantial toxicity.
The latest treatment guideline in 2014 recommends treating all HIV-1 infected patients regardless of their CD4 count. Benefits of early successful viral suppression outweigh the potential toxicity that has become manageable with newer drugs. Two major effects can be achieved if patients are treated early. On the individual level, a high CD4 count is associated with better survival in general in HIV-infected patients. On the population level, HIV-1 transmission can be strongly reduced because if viral load is suppressed an HIV-1 infected individual is not infectious anymore [45, 144, 145]. Of course most importantly, the patients' willingness to adhere to treatment is the prerequisite for any successful antiretroviral treatment [136].

3.5 Treatment efficacy

Overall, given the number of antiretroviral agents and different drug classes available today, most patients, including those having failed treatments and even carrying multiclass drug resistance, can still successfully achieve viral suppression [53]. However, treatment efficacy can be violated if drug concentration drops below the effective level. This is usually influenced by non-adherence, including missing or delaying a dosage, or drug interactions with another antiretroviral agent or other drugs [146]. For checking drug interactions a comprehensive database can be found at: `http://www.hiv-druginteractions.org/`. Clinicians familiar with antiretroviral therapy generally pay attention to this matter and try to avoid possible drug-drug interactions before subscribing a treatment. For adherence issues, several studies have focused on understanding the changing behavior of adherence for possible future prevention [147–150]. One of the predictors for suboptimal adherence is higher pill burden. Recently, a meta-analysis has demonstrated an association of lower pill burden with better adherence [151]. On the other hand, due to the upcoming availability of generic drugs it is possible that insurance companies and third-party payers are more willing to pay for generic drugs because they cost less than co-formulations. One line of my research focused on comparing the relative efficacy of four mostly used NRTI backbones over time while taking into account the different number of pills (see Research Project 3 [Chapter 10] for more on this topic).

4 HIV-1 Drug Resistance

One of the major concerns of treating any infection is the emergence of drug resistance against the antimicrobial agents used (true for bacteria, mycobacteria, parasites, fungal and viral infections). *In vitro* and *in vivo* drug resistance was selected against all available antiretroviral drugs developed for treating HIV-1 [152]. Although more than 25 antiretroviral agents are available for treating HIV-1 infection, broad cross-resistance within drug classes and low genetic barriers of many drugs may decrease options for subsequent treatments considerably.

The genetic barrier is defined as the number of mutations a virus needs to accumulate to become resistant to a given antiretroviral drug. For example, EFV from the NNRTI class has a low genetic barrier because the virus only requires one amino acid mutation (e.g. the K103N) to confer full resistance to the drug. On the contrary, an antiretroviral agent of high genetic barrier would not lose its activity until multiple mutations have been accumulated, e.g., the boosted protease inhibitors.

Unfortunately, many agents with low genetic barriers also select for mutations that confer broad cross-resistance, i.e., resistance mutations acquired from an agent confers resistance against other agents. Key mutations such as K103N and Y181C alone can already lead to inactivity of almost all NNRTIs [153]. Among NRTIs, a number of different mutations are often selected upon failure and cause loss of activity to many other NRTIs [153, 154]. Compared to NRTIs and NNRTIs, boosted PIs (PI/r) have higher genetic barriers [155] and so does the newest integrase inhibitor dolutegravir [156, 157]. Even with long exposure to failing cART, PI/r containing regimens induce lower frequencies of PI mutations and also prevent NRTI resistance when compared to NNRTI containing regimens [158].

On the other hand, some drug resistant mutations do not always decrease the susceptibility of a virus to all drugs; some mutations even increase the susceptibility to other drugs. M184V is an example: M184V can be selected by ABC, FTC, or 3TC but at the same time increases the susceptibility to AZT, TDF, and d4T [159–163].

There are several web resources for defining resistance mutations and predicting drug susceptibility based on expert opinions. Among others, the Stanford drug resistance database (`http://sierra2.stanford.edu/sierra/servlet/JSierra`) provides useful online tools for genotype resistance interpretation using their own algorithm, based on published phenotypic studies and repeated testing. The Stanford resistance interpretation tool gives a penalty score for submitted sequences to predict the drug susceptibility with given mutations. In addition to the Stanford algorithm, other genotypic resistance interpretation systems have also been widely accepted and used: for example from the Rega institute (`http://rega.kuleuven.be/cev/`) and from the Agence Nationale

de Recherches sur le SIDA (ANRS; `http://www.medpocket.com/`) [164]. Advanced statistical models and machine-learning algorithms based on data retrieved from viral sequences and phenotypic assays are also being developed to predict treatment failures [165].

4.1 Genetic variability

Due to the nature of an error-prone reverse transcriptase, it is estimated that the mutation rate of HIV is 1.4-3 x 10^{-5} mutations/bp/cycle [166, 167]. Given that the HIV genome is $\approx$ 10000 bp, one mutation is synthesized in every 10 replication cycles. This is within the average range of the retroviral mutation rate. However, as pointed out by Coffin et al. [168], the genetic diversity of HIV-1 in patients is not only owing to errors made during the viral replication but also to the high rate of viral replication and the high viral load. Given that an average HIV plasma viral load in an untreated patient is $\approx 10^5/m\ell$, every day $\approx 10^{10}$ virions are estimated to be produced [167, 169, 170]. These factors combined with recombination, which can occur when a cell is co-infected with two or more divergent HIV strains, lead to a high diversity of HIV variants [171, 172]. In theory, every position of the HIV genome in an individual can be mutated every day [168, 173]. This means that variants harboring drug resistant mutations are constantly generated also in the absence of drugs. Among the large number of variants generated per day, many variants with lethal mutations simply die out soon after being produced. Those variants that can replicate in the normal host environment, i.e., in the absence of the selection pressure of antiretroviral drugs, are called the wild type, to distinguish them from other variants harboring mutations that confer drug resistance.

4.2 Selection of drug resistance

In the case of HIV, drug-resistant variants are selected by drug pressure exerted by antiretroviral drugs. In other words, drug-resistant mutants are more fit in the environment with drug pressure when compared to wild type variants. The drug-resistant mutants usually harbor mutations that contribute to modifications on important drug targeted enzymes such as reverse transcriptase, protease, and integrase to bypass the antiretroviral activity of a drug, which enables them to continue to replicate under drug pressure. Due to the high turn over rate of HIV, drug-resistant variants can outgrow wild type populations quickly and become the majority in the quasispecies within days to weeks [170, 174, 175]. The reason for the selection of specific variants in a given environment is that some variants have the ability to replicate more effectively than others, a property named fitness [38]. For example, mutations conferring resistance to NRTIs allow viruses to replicate in the presence of NRTIs, but without such drugs these mutations actually decrease the viral replication capacity. In other words, mutations can come with a fitness cost. Fitness cost of a specific mutation or of a combination of mutations vary, depending largely on the viral genetic background modulated by compensatory mutations [176–178].
In Research Project 2 (Chapter 9), the reversion rates and fitness costs of various transmitted drug resistance mutations were studied in detail *in vivo*.
As mentioned before, factors required for creating variations including mutations are: high replication rate, high error rate, high viral load, and recombination [168, 179, 180]. As a result, drug resistance will rarely emerge if one of the factors is missing. When viremia is controlled and suppressed by therapy, the virus does not replicate and thus no viral evolution is occurring. Thus, in this perfect setting there is hardly any chance for drug

resistance to emerge. Unfortunately, such a condition is usually not achieved in resource-limited settings for a large fraction of patients. Due to non-adherence, drug shortage, and lack of monitoring, drug resistance continues to emerge in these settings.

4.3 Mechanisms of drug resistance

In this section the resistance mechanism of the major three drug classes is summarized.
NRTI resistance includes two mechanisms: 1) the removal of the incorporated NRTI, and 2) the prevention of the incorporation of NRTI due to increased discrimination between natural nucleotides and nucleos(t)ide analogues.
The first mechanism is related to thymidine analogue mutations (TAMs; TAM 1 includes M41L, L210W, and T215Y, and TAM 2 includes D67N, K70R, T215F, and K219Q/E) and is involved in the ATP-mediated removal of AZT [181–184], d4t and ddI [185, 186] from the 3' end of the DNA chain. ATP, which is abundant in lymphocytes, does not participate in the process of DNA polymerization generally, but TAMs cause a conformational change of reverse transcriptase, leading it to be able to bind ATP [187, 188]. ATP can thus attack the bond between NRTI and DNA and facilitate the removal of NRTI. The second mechanism that prevents NRTI from incorporating into the DNA chain is mediated by mutations such as M184V/I, Q151M, and K65R. This group of mutations cause changes to the reverse transcriptase so that the ability to incorporate NRTIs instead of natural nucleotides is decreased [189]. The mutation M184V/I is selected by 3TC and FTC, and less frequently by ABC. The position 184 is located in the middle of the catalytic site of the reverse transcriptase [190], and the replacement of a methionine by a valine perturbs the binding to 3TC, FTC, or ABC, resulting in the inhibition of those drugs. On the other hand, it was shown that mutation M184V in the presence of ATP decreases the ability to remove AZT [191]. Q151M is actually a group of mutations with Q151M being always the first one to emerge in the group, and is also called the 151 complex. This pathway starts with the substitution at the position 151 and is followed gradually by secondary mutations (among others the A62V, V75I, F77L, F116Y) [192]. The 151 complex affects all NRTIs currently approved except tenofovir [193, 194]. The mutation K65R is located in the nucleotide-binding pocket and perturbs the binding activity to most NRTIs except AZT [195].
Resistance mutations to NNRTI are all located in the catalytic binding site targeted by NNRTIs and reduce the affinity of NNRTIs to the reverse transcriptase [196, 197]. Although this drug class is affected strongly by cross-resistance between different agents, the most possible selection of a point mutation is dependent on the drug. For example, the mutation Y181C is often associated with the use of NVP [198], and the K103N mutation is mostly seen in patients failing EFV containing regimens [199]. Etravirine, being the 2nd generation of NNRTI, is fully active against viral isolates harboring the K103N mutation. However, activity vanishes rapidly if other NNRTI associated resistance mutations are present [152]. Rilpivirene is the latest NNRTI and shows cross-resistance to almost all major NNRTI mutations [152].
For PI resistance, primary mutations (also called major mutations) often emerge first, followed by accumulation of secondary mutations (or minor mutations) [200, 201], which are often already present as polymorphisms regardless of therapy. The presence of a large number of polymorphisms is a specific feature of the protease gene; however, it was shown that these polymorphisms are not relevant for treatment failure in patients starting PI based regimens in subtype B infected patients [202]. Primary PI mutations are located near the enzyme's binding site, which is the same site binding both inhibitors and the natural protease substrate, Gag and Gag-Pol polyproteins. Thus, PI resistance distorting

the domain flexibility of the binding site reduces not only the binding to inhibitors but also the natural substrates, and so reduces the viral replication fitness [203]. With time the virus generates and selects minor mutations that directly compensate for the viral fitness reduced by major mutations [177, 204, 205]. After the accumulation of secondary mutations, further mutations can be selected to create a novel Gag-Pol frame shift site, resulting in more Gag-Pol polyproteins being produced [206]. This further compensates the reduced fitness caused by major mutations. Taken together, HIV utilizes this stepwise mutation pathway to escape from the PIs and at the same time to restore the viral fitness [204].

4.4 Acquired drug resistance

If drug-resistant variants did not exist in a patient at the time when this person was infected and were selected during the course of treatment through the exerted drug pressure, this kind of drug resistance is referred to as acquired drug resistance. As the name suggests, the resistance in general is acquired on a failing, or not fully active, treatment.

When treatment is discontinued, the selection pressure of drugs disappears gradually depending on the half-life of the drugs. The different half-lives play an important role for emergence of acquired drug resistance when stopping therapy. For example, EFV or NVP usually have long half-lives compared with most NRTIs. If all drugs in a regimen containing NNRTI and NRTIs are stopped at the same time, levels of NNRTI last longer than NRTIs leaving a period of monotherapy with functional NNRTI only. This may select NNRTI resistance [207]. After discontinuation of therapy, the virus with detectable drug resistance can soon become susceptible again [99, 208] because the wild type viral strain that circulated before the initiation of the therapy has been archived in the latent reservoir (see Chapter 2.3 for latency) [85, 97, 209, 210], and becomes again the fittest variant in the quacispecies. The reversion rate of a drug resistance mutation also depends on the differential fitness costs of mutations [211]. In general, we define reversion of a mutation as decreasing to below the detection limit of population sequencing, which has changed over time (from the initial detection limit of 400 copies/$m\ell$ to 50 copies/$m\ell$, and now to 20 copies/$m\ell$).

A persisting or a relapsing HIV viral load in a treated patient is an indicator for treatment failure and often resistance emerges in those patients. To test which drug resistance mutations have been acquired, one needs to perform resistance testing. It remains a challenge to appropriately interpret results of resistance testing. Predefined lists are created for this purpose, and I consulted the IAS-USA surveillance list for defining acquired drug resistance mutations [152, 193]. More details about resistance testing are given in Chapter 4.6.

4.5 Transmitted drug resistance

Patients on a failing treatment are potentially infectious with drug-resistant viruses. If such patients infect other healthy individuals or in rare cases also superinfect an already HIV-infected individual [212], the newly infected patient will unavoidably carry the drug-resistant viruses without ever having been exposed to antiretroviral treatment. This kind of resistance is then referred to as transmitted drug resistance.

Transmitted drug resistance in most cases is carried by treatment-naïve individuals. As

opposed to acquired drug resistance, transmitted drug resistance can persist longer in an environment without drug pressure, due to the fact that in 60-90% of cases only one variant from a transmitter is transmitted [45, 46] and thus in those being infected with a drug-resistant variant, the drug-susceptible wild type is not transmitted to the recipient and cannot be archived in the latent reservoir. To acquire the wild type variant, the virus needs to mutate at the amino acid position associated with drug resistance. It is generally assumed that the wild type emerges at a different pace depending on the fitness cost that the mutation is associated with [146]. It will do so rapidly only if a fitness benefit occurs with reversion of drug resistance mutations. Previous findings have shown that the differential persistence time of transmitted drug resistance mutations vary considerably [213–215]. Moreover, after reversion, transmitted drug resistance mutations persist as a minority [216] and can jeopardize the first-line treatment efficacy [174, 217–219]. It has also been unambiguously shown by our group [220] that minority variants carrying drug resistant mutations can be transmitted, but to date it remains controversial how important these minority variants are for treatment success [217, 221, 222]. They are most likely not relevant for high genetic barrier drug treatment [216].
I have studied in detail the relationship of reversion with fitness cost of a mutation in Research Project 2 (Chapter 9), and the prevalence of transmitted drug resistance in Research Project 1 (Chapter 8). For my projects about TDR, I consulted the WHO surveillance list for transmitted drug resistance to define mutations [223, 224].

4.6 Resistance testing

To test if a patient harbors drug resistance it is necessary to perform resistance testing at several time points. Today guidelines [146] recommend resistance testing for both treatment-naïve patients and treatment-experienced patients. To identify whether a newly infected patient harbors transmitted drug resistance, all treatment-naïve patients are recommended to have resistance test at entry into care regardless of whether cART will be initiated immediately or not [146]. The idea of early resistance testing is to identify as many resistance mutations as possible including the fast-reverting mutations such as M184V and K65R [214, 225, 226] to prevent early treatment failure [174, 216]. When changing a regimen, resistance tests can assist the selection of subsequent drug regimens. Therefore, it is especially recommended to perform a resistance test at the time of changing a regimen in patients changing due to treatment failure. For patients on treatment whose viral load cannot be suppressed, it is also recommended to test resistance to manage suboptimal viral load reduction [146].
There are two types of assays to test drug resistance: phenotypic and genotypic assays. The tested gene of interest is the gene that the drug exerts selection pressure on, e.g. part of the *pol* gene for PIs, NRTIs, NNRTIs, and integrase inhibitors, and part of the *env* gene for Maraviroc, etc.

4.6.1 Genotypic resistance test

The genotypic resistance assays sequence viral genes using population sequencing techniques and predict the virus's susceptibility of antiretroviral drugs based on predefined master lists [152, 193, 224] or rule-based algorithms [164]. Due to the low cost and short performance time, genotypic testing is preferred for routine clinical practice.
Since genotypic resistance testing requires expert interpretations of the results, the major limitation of it is to correctly predefine mutations. This can be challenging because the impact of different mutations on drug susceptibility varies largely. Predictions of

available algorithms are not always in agreement, which makes interpretation of genotypic resistance tests sometimes difficult [146, 227].

4.6.2 Phenotypic resistance test

Phenotypic testing today mostly uses recombinant virus assays based on the construction of a reference HIV vector with inserted genes of patiens' viruses. Theses recombinant viruses are cultured in the presence of a single drug at different concentrations. The fold-change of the amount of drug needed to inhibit viral replication by 50% (called the half maximum inhibitory concentration (IC_{50})) is measured. Phenotypic assays can yield direct results for patients individually, and no predefined master lists for testing interpretation are needed [146, 227]. However, to perform phenotypic testing it takes more time and more money compared to genotypic testing. In addition, comparative clinical studies have never shown superiority of phenotypic assays over genotypic tests [228, 229]. Therefore, phenotypic resistance tests are mainly used for studying emergence of resistance against new drugs *in vitro* and in the first clinical studies.

4.7 Resistance prevalence

Drug resistance can be found in every country where antiretroviral drugs are available. The continuing evolution of drug resistance in circulating HIV variants presents a global challenge.

In resource-rich countries, drug resistance is mostly under control. Prevalence of acquired drug resistance has declined dramatically due to successful treatments and regular monitoring [132, 230], and prevalence of transmitted drug resistance has remained stable, at around 8% to 10%, over time [31, 231–234]. Among drug classes, transmitted NRTI resistance has the highest prevalence, which has been stable or even decreasing over time. On the contrary, transmitted NNRTI resistance has the lowest prevalence compared to NRTIs and PIs but has risen in the past years [235]. Two conclusions can be drawn here. On the one hand, the different time trends between drug classes suggest that prevalence of transmitted drug resistance is associated with the roll-out time of antiretroviral drugs. NNRTI being the last to be introduced among the three major drug classes expectedly has the lowest prevalence that is increasing, but eventually the increase will stop as seen in the prevalence of transmitted NRTI resistance and it was predicted by mathematical modeling by Blower et al. that prevalence of transmitted drug resistance will remain low because its increase reaches a plateau [236]. On the other hand, if drug resistance is primarily transmitted by treatment-failing patients as generally hypothesized, the constant decrease of the rate of acquired drug resistance and the stable, or in some countries the increasing, trend of the rate of transmitted drug resistance together present a paradox. To further assess this paradox was exactly the aim of my Research Project 1 (Chapter 8).

For resource-limited countries, situations are different. Although prevalence of transmitted drug resistance is lower in resource-limited countries [237, 238], it was found to have increased substantially since the roll-out of antiretroviral drugs in individual countries [239–241]. Two potential dangers exist. First, rising prevalence of transmitted drug resistance could increase both numbers of early first-line treatment failures [218] and prevalence of multiclass resistance. Since drug options are limited in these settings and viral load monitoring is still the exception, controlling the epidemic there could presumably become a problem in the long term. Secondly, since in high-income countries there is a lot of traveling to resource-limited countries (e.g., business, vacation, sex tourism) and immigration

from resource-limited countries, in particular to Europe, are strongly increasing, more new infections harboring transmitted drug resistance might be expected in high-income countries in the long run.
In conclusion, drug resistance still represents a global concern. To develop a timely strategy to restrain both acquired drug resistance and the transmitted drug resistance, which has earned rising attention, one needs to first understand the mechanism of how drug resistance spreads.

5 The Swiss HIV Cohort Study (SHCS) and Databases

All my research projects are based on information stored either in the Swiss HIV Cohort Study (SHCS) database or the SHCS drug resistance database.

5.1 The SHCS

The SHCS (`www.shcs.ch`) is a prospective and nationwide cohort study including a large biobank consisting of plasma and liquid nitrogen frozen cells. It continuously enrolls patients of all transmission groups aged 16 or older since 1988. The study is a collaboration of seven large medical centers (including all 5 Swiss University hospitals): Basel, Bern, Geneva, Lausanne, Lugano, St. Gallen, and Zurich. In addition, smaller regional hospitals and private physicians carrying for HIV patients are associated with the large centers. The SHCS has been approved by ethical committees of all participating institutions. Written informed consent was obtained from all patients. Basic information of patients such as gender, birth, and ethnicity are collected at registration [10, 242], and clinical data such as CD4 count and HIV-RNA viral load are collected every six months at each visit. In addition, detailed treatment history including product name and dosage is documented, and a systematic questionnaire is conducted addressing, e.g., drug adherence, toxicity, living conditions, and sexual behavior.
The SHCS is representative for the HIV epidemic in Switzerland. The SHCS includes at least 53% of all HIV cases ever diagnosed, 72% of all patients receiving ART, and 69% of the nationwide registered AIDS cases in Switzerland [242]. In fact, these numbers are based on a conservative estimate. For example, the Swiss Federal Office of Public Health (FOPH) estimated that around 500 - 1000 people were newly diagnosed with a HIV-1 infection each year from 1996 to 2012, resulting in 11870 cases in total. This was in approximate accordance to the number of participants enrolled into the SHCS during the same time period (n=10050 from 1996-2012; Figure 5.1). Thus, from 1996 onward to at least 2012, the coverage of the SHCS for the Swiss epidemic is $\approx 85\%$.
Up to February 2015, the SHCS has enrolled 18734 patients and $\approx 81\%$ of patients have received treatment.

5.1.1 The Zurich Primary HIV Infection (ZPHI) Study

The Zurich Primary HIV infection study (ZPHI:`http://www.viralinfectiousdiseases.uzh.ch/ZPHI.html`) was initiated in 2002. Since 2012, the ZPHI belongs to the Clinical

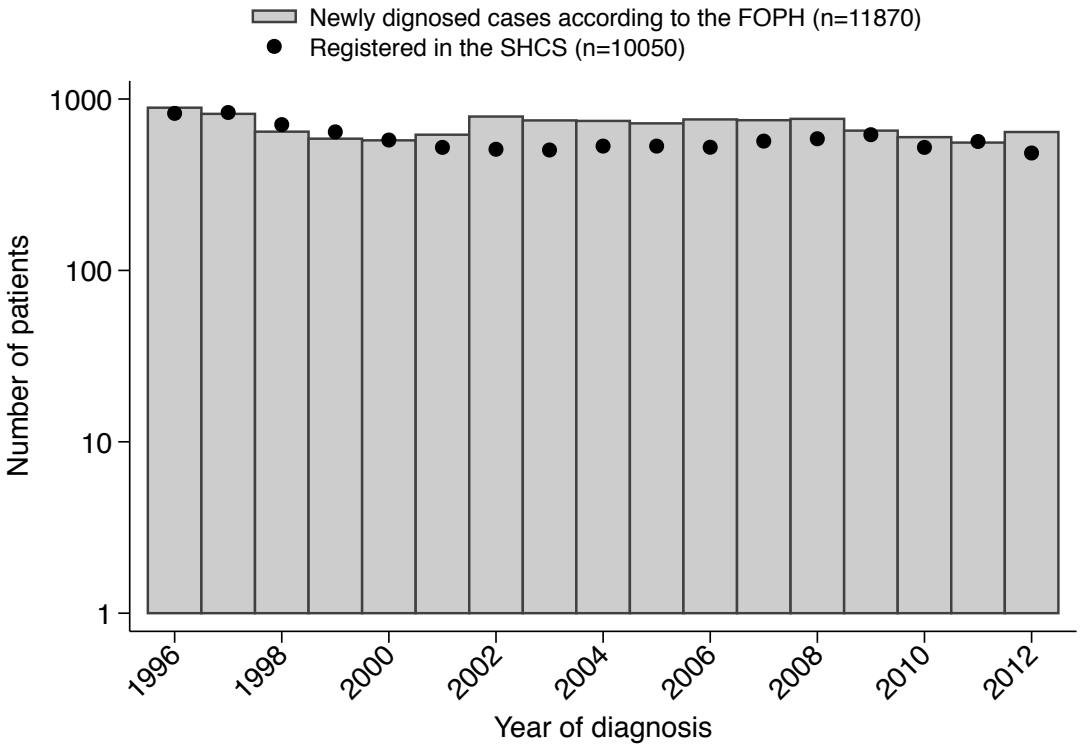

Figure 5.1: Representativeness of the SHCS

Research Priority Program (CRPP) Viral Infectious Diseases, which is a research project funded by the University of Zurich. The ZPHI enrolls patients with primay HIV-1 infection. Most of the patients are also enrolled in the SHCS. The focus of ZPHI is to explore new treatment strategies in HIV-1 primary infected patients.

5.2 The SHCS drug resistance database

The SHCS drug resistance database collects all genotypic resistance tests performed for SHCS patients in clinical routine and for research purposes. Four laboratories in Switzerland are authorized by the Federal Office of Public Health to perform these tests. All laboratories perform population-based sequencing of the full protease gene and at least codons 28-225 of the reverse transcriptase gene using either in-house methods [243] or commercial assays (Viroseq Vs.1 PE Biosystems; Virsoseq Vs.2, Abbott AG; VircoTYPE HIV-1 Assay, Virco Lab). In addition, all laboratories have participated in the annual quality control evaluation by the Agence Nationale de la Recherche du SIDA (ANRS) since 2002. All sequences are entered into the SHCS drug resistance database using SmartGene's Integrated Dababase Network System (SmartGene, Zug, Switzerland, IDNS version 3.6.3) [244]. A large part of sequences generated retrospectively was selected for my PhD work (for more detail see Chapter 7). In addition to resistance mutation data and all essential test information such as sequence identifier and sequence date, subtypes are also stored in the database. Subtyping is performed on the protease and the reverse transcriptase sequence using REGA 2 (`http://jose.med.kuleuven.be/genotypetool/`

`html/subtypinghiv.html`). If it returns inconclusive results, the analysis is repeated with the Star analyzer (`http://www.vgb.ucl.ac.uk/starn.shtml`) [245].
Up to June 2014, there were 9764 sequences from 4932 treatment-experienced patients and 10680 sequences from 8089 treatment-nave patients stored in the database.

6 Thesis Objectives

My research focused on several aspects that are fundamental to achieving treatment success. In the first project I studied the paradox between the prevalence of acquired and transmitted drug resistance as well as time trends of prevalence and associated risk factors. In the second project I analyzed the time needed for reversion of transmitted drug resistance mutations and its association with the fitness cost of mutations. In the third project I compared the efficacy of four most used and recommended NRTI combinations in a NNRTI backbone taking into account the different numbers of pills and dosing frequencies of the regimens.
The aim of these projects was to provide additional helpful information to better understand the interplay between transmitted and acquired drug resistance on the population level and to help to improve the outcome of antiretroviral treatment in the long run. Furthermore, I believe that my results will also be of value for public health policies in Switzerland and abroad.

II

Research Projects

7 Sample Selection for Retrospective Sequencing

The SHCS resistance database consists of genotypic resistance tests performed for both clinical routine and research purposes. Most tests for research purposes were performed retrospectively, especially for patient plasma samples originating from before 2002 when genotyping was not yet integrated into routine clinical care.
An essential amount of time for this PhD thesis was dedicated to systematically identify plasma samples according to a variety of criteria for retrospective sequencing. Samples were retrieved from separate freezers located in seven clinical centers throughout Switzerland and sent to the sequencing laboratories of the Institute of Medical Virology, University of Zurich, and Laboratory of Virology, Geneva University Hospital. In total, 5464 samples were found to complement the SHCS resistance database in the following ways:

1. to have a baseline sequence for every treatment-naïve patient (n=1530 for this purpose)

2. to have sequential sequences from every treatment-naïve patient carrying transmitted drug resistance before starting treatment (n=944)

3. to have a genotyped sequence for every failed treatment per patient (n=2017)

4. to have an integrase sequence for treatment-naïve patients registered after Jan. 1, 2009 and treatment-failing patients who have failed for the first time after Jan. 1, 2009 (n=819)

5. to study subtype evolution until undergoing treatment, i.e., to have sequential sequences from patients infected with one of the most frequent subtypes in the SHCS: B, A, C, AE, AG (n=990)

8

Assessing the Paradox Between Transmitted and Acquired HIV-1 Drug Resistance in the Swiss HIV Cohort Study From 1998 to 2012

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Personal Contributions

For this study I systematically selected patient plasma samples for retrospective sequencing. First, I looked for patients without a baseline genotypic sequence while being treatment-naïve and patients failing a treatment who had not been sequenced. Subsequently, I searched in the SHCS database if these patients have suitable and available plasma samples stored in the SHCS biobank. If yes, I requested them to be retrieved and retrospectively sequenced. I combined the genotyping information from the SHCS resistance database with the treatment information from the SHCS databases, selected patients that matched the criteria of our study aim and documented every inclusion and exclusion step including reasons and numbers yielded. I did all statistical analyses. I generated and designed all figures and tables. Finally, I wrote the first version of the manuscript and edited it after comments of the co-authors.

Abstract

Background
Transmitted HIV-1 drug-resistance mutations (TDR) are transmitted from treatment-failing or treatment-naïve patients. Although prevalence of drug-resistance in treatment-failing patients has declined in developed countries, TDR prevalence has not. Mechanisms causing this paradox are poorly explored.
Methods
We included recently-infected, treatment-naïve patients with genotypic-resistance-tests performed ≤ 1 year post-infection and < 2013. Potential risk factors for TDR were analyzed using logistic regression. Association of TDR prevalences with population viral load(PVL) from treatment-patients during 1997 - 2011 was estimated with Poisson regression for all TDR and individually for most frequent resistance-mutations against each drug class (M184V/L90M/K103N).
Results
We included 2421 recently-infected, treatment-naïve patients and 5399 treatment-failing patients. TDR prevalence fluctuated considerably over time. Two opposing developments could explain these fluctuations: generally continuous increases in TDR (Odds Ratio [OR] $= 1.13$, $p = 0.010$), punctuated by sharp decreases when new drug-classes were introduced. Overall, TDR prevalence increased with decreasing PVL (Rate Ratio[RR] $= 0.91$ per 1000 increase in PVL, $p = 0.033$). Additionally, we observed that the transmitted high-fitness-cost mutation M184V was positively associated with PVL of treatment-failing patients carrying M184V (RR $= 1.50$ per 100 increase in PVL, $p < 0.001$). Such association was absent and negative for K103N (RR-K103N $= 1.00$ per 100 increase in PVL, $p = 0.99$) and L90M (RR-L90M $= 0.75$ per 100 increase in PVL, $p = 0.022$), respectively.
Conclusions
Transmission of antiretroviral drug-resistance is temporarily reduced by the introduction of new drug classes and driven by treatment-failing and treatment-naïve patients. These findings suggest a continuous need for new drugs, early detection and early treatment of HIV-1-infection.

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MAJOR ARTICLE

Assessing the Paradox Between Transmitted and Acquired HIV Type 1 Drug Resistance Mutations in the Swiss HIV Cohort Study From 1998 to 2012

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Background. Transmitted human immunodeficiency virus type 1 (HIV) drug resistance (TDR) mutations are transmitted from nonresponding patients (defined as patients with no initial response to treatment and those with an initial response for whom treatment later failed) or from patients who are naive to treatment. Although the prevalence of drug resistance in patients who are not responding to treatment has declined in developed countries, the prevalence of TDR mutations has not. Mechanisms causing this paradox are poorly explored.

Methods. We included recently infected, treatment-naive patients with genotypic resistance tests performed ≤1 year after infection and before 2013. Potential risk factors for TDR mutations were analyzed using logistic regression. The association between the prevalence of TDR mutations and population viral load (PVL) among treated patients during 1997–2011 was estimated with Poisson regression for all TDR mutations and individually for the most frequent resistance mutations against each drug class (ie, M184V/L90M/K103N).

Results. We included 2421 recently infected, treatment-naive patients and 5399 patients with no response to treatment. The prevalence of TDR mutations fluctuated considerably over time. Two opposing developments could explain these fluctuations: generally continuous increases in the prevalence of TDR mutations (odds ratio, 1.13; $P = .010$), punctuated by sharp decreases in the prevalence when new drug classes were introduced. Overall, the prevalence of TDR mutations increased with decreasing PVL (rate ratio [RR], 0.91 per 1000 decrease in PVL; $P = .033$). Additionally, we observed that the transmitted high-fitness-cost mutation M184V was positively associated with the PVL of nonresponding patients carrying M184V (RR, 1.50 per 100 increase in PVL; $P < .001$). Such association was absent for K103N (RR, 1.00 per 100 increase in PVL; $P = .99$) and negative for L90M (RR, 0.75 per 100 increase in PVL; $P = .022$).

Conclusions. Transmission of antiretroviral drug resistance is temporarily reduced by the introduction of new drug classes and driven by nonresponding and treatment-naive patients. These findings suggest a continuous need for new drugs, early detection/treatment of HIV-1 infection.

Keywords. HIV; transmission; drug resistance; recently infected; fitness.

Transmission of human immunodeficiency virus type 1 (HIV-1) infection depends strongly on individual levels of plasma viremia [1]. When HIV-1–infected patients

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8.1 Introduction

Transmission of HIV-1 infection depends strongly on individual levels of plasma viremia [246]. When HIV-1-infected patients receive suboptimal treatment or incomplete adherence to anti-retroviral therapy (ART), drug-resistant viruses emerge and continue replicating. Therefore, the general assumption is that drug-resistant viruses are mainly transmitted from treated patients with high levels of HIV viremia due to failed [**Hirsch:1998wb**]. Modern ART reduces the viremia levels and transmissibility of HIV-1 more effectively than earlier ART [247], suggesting less emergence [133] and transmission of HIV-1 drug-resistance over time.
In recent years, the incidence and prevalence of acquired drug-resistance mutations (ADRs) in treated patients has indeed declined due to effective ART in various developed countries [132, 230]. However, prevalence of transmitted drug-resistance mutations (TDR) has often remained stable [231, 235, 248]. TDR may cause early virological failure when patients start their first-line therapy [218]. Certain TDR can persist for years in the absence of drug pressure after seroconversion [214] and have long-term potential to jeopardize the effectiveness of ART; other TDR may disappear rapidly and become undetectable via population sequencing [214, 217]. Recently, transmission of minority variants harboring drug-resistance has been demonstrated [220]. Difficulties in detecting TDR upon ART initiation might therefore compromise the treatment success achieved thus far.
In the current study we aimed at analysing the risk factors of TDR, and resolving the discrepant patterns of TDR and ADR prevalence over time. The unique SHCS dataset, which is representative for $\geq$ 15 years, allows us to determine the impact of temporarily changing factors such as numbers of available drug classes. We adapted population viral load (PVL) as a tool to assess the spread of drug-resistance and the transmission potential of the treatment-experienced population. We focused specifically on TDR during recent infections to avoid potential bias caused by different TDR persistence times.

8.2 Methods

Study population

The SHCS, enrolling patients since 1988, is a prospective, nationwide, clinic-based study including a biobank. The SHCS is representative for the HIV epidemiology in Switzerland; it includes at least 53% of all HIV cases ever diagnosed in Switzerland, 72% of all patients receiving ART, and 69% of the nationwide registered AIDS cases [242]. Additionally, we enrolled patients from the Zurich Primary HIV-infection study (ZPHI:`www.clinicaltrials.gov`; ID=NCT00537966) which focuses on identifying and treating patients during early infection [46]. Ethical approval from all participating institutions and written informed consent from all patients was obtained [10, 46, 242].
To identify the TDR prevalence, we included recently-infected, treatment-naïve patients (definition: see below) with a genotypic resistance test (GRT) performed before 1.1.2013. The first GRT from each recently-infected, treatment-naïve individual was considered. All sequences before 1996 were grouped as $\leq$ 1995 because of small sample sizes. For the association analysis, in which we tested whether TDR prevalence is associated with the PVL from ART-failing patients, we included ART-failing patients from 1997 - 2011 due to the representative availability of VL testing since 1997.
GRTs stem from routine-clinical testing performed by four laboratories in Switzerland authorized by the Federal Office of Public Health. All laboratories perform population-based sequencing of the full protease gene and at least codons 28 - 225 of the reverse

transcriptase gene using commercial assays (Viroseq Vs.1 PE Biosystems;Virsoseq Vs. 2, Abbott AG; vircoTYPE HIV-1 Assay, Virco Lab) and in-house methods [243] and participate in the yearly quality control evaluation by the Agence Nationale de la Recherche du SIDA (ANRS) since 2002. All sequences are entered into the SHCS drug-resistance database using SmartGene's Integrated Dababase Network System (SmartGene, Zug, Switzerland, IDNS version 3.6.3) [244]. Additionally, we performed systematically retrospective sequencing for blood samples that were stored in the biobank before routine genotyping was introduced (over 11000 sequences were retrospectively generated). Subtyping was performed on the protease and the reverse transcriptase sequence using REGA 2 (`http://jose.med.kuleuven.be/genotypetool/html/subtypinghiv.html`). If this method returned inconclusive results, the analysis was repeated with the Star analyzer (`http://www.vgb.ucl.ac.uk/starn.shtml`) [245].
TDR were identified using the WHO list for surveillance of transmitted HIV drug-resistance [224].

Definition of recent infection

To account for potential reversion of TDR in the absence of drug pressure [**Devereux:1999ua**, 213, 214, 225, 249, 250], we restricted our study population to treatment-naïve patients having been diagnosed ≤ 1 year after infection. Specifically, we determined recent infection with one of the following methods:

1. Documented acute HIV-1 infection as previously described [46].

2. Documented seroconversion (< 1 year between the last negative and first positive HIV tests).

3. For those lacking the data mentioned above, the ambiguity score [49] was used. It is a measure of the viral nucleotide diversity from bulk sequencing which estimates the infection duration. Sequences with $\leq 0.5\%$ ambiguous nucleotides were considered to be GRTs from recently-infected patients [49]. However, as diversity may be low in long-term HIV-infections, patients with a score $\leq 0.5\%$ and a CD4 count < 200 were excluded to reduce false positives. For validation of this method, see Supplementary Material.

The viral burden of treatment-failing patients

PVL was used to describe the viral burden of ART-failing patients for the coming year on a population level. We summed up the $\log_{10}$ transformed VLs from all ART-failing patients of a given year. For further analyses, where we studied the transmission pattern of a specific TDR, the total of $\log_{10}$ transformed VLs from ART-failing patients carrying the corresponding mutation was used. Only VLs corresponding to a GRT were included for these analyses because genotyping was needed to determine drug-resistance mutations. To acquire all potential treatment failures, we defined treatment failure as having a VL $\geq$ 400 copies/$m\ell$ after 180 days of continuous ART. VL measurement was not fully integrated into the clinical routine before 1997, so we included VLs from treatment-failing patients during 1997 - 2011. Each person contributed to each year once. If a patient had ≥ 2 VLs measured within the same year, we calculated the mean for that year.

Statistical methods

Potential risk factors for acquiring any TDR were analyzed using logistic regression. Variables investigated were ethnicity (Caucasian, Black, others), gender (male, female), transmission group (men having sex with men [MSM], heterosexual transmission [HSX], injecting drug users [IDU], others), HIV-1 subtype (B, non-B), and the calendar year of sampling (fitted as a continuous variable). Additionally, since we suspected that less optimal regimens resulting from fewer choices of available drugs might have influenced TDR transmission, we included the number of available drug classes as an ordered categorical variable (the p-value was obtained from the test for trend). In Switzerland, HIV-1 treatment occurred in five eras, each separated by the introduction of a new drug class: Mono-class therapy with nucleoside analogue reverse transcriptase inhibitors (NRTI) was used before 1996 (1 drug class:$\leq$ 1996). After the introduction of unboosted protease inhibitors (PI) in 1996, patients could obtain dual-class regimens (2 classes:1997 - 1998). Subsequently, non-nucleoside analogue reverse transcriptase inhibitors (NNRTI) were introduced in 1998 (3 classes:1999 - 2000), followed by boosted PI (PI/r) in 2000 (4 classes:2001 - 2008), and integrase inhibitor (InSTI) in 2008 (5 classes:2009 - 2012). In the model we included binary response indicating detection of any TDR from each patient as an outcome. We analyzed variables independently and included those associated significantly with the outcome into the multivariable model (HIV subtype and transmission group). We also chose variables a priori regardless of univariable significance due to likely biological impacts (sex, year, and number of available drug classes). For TDR to individual drug classes, we included the same co-variables in the multivariable models for reasons of consistency to avoid obtaining a different set of variables for each drug class. We found no collinearity and interactions between any included variable. Missing data were list-wise deleted. We calculated odds of TDR detection from our fitted multivariable model by retaining all co-variables except for year and number of available drug classes at baseline, and transformed the predicted odds to annual prevalences.

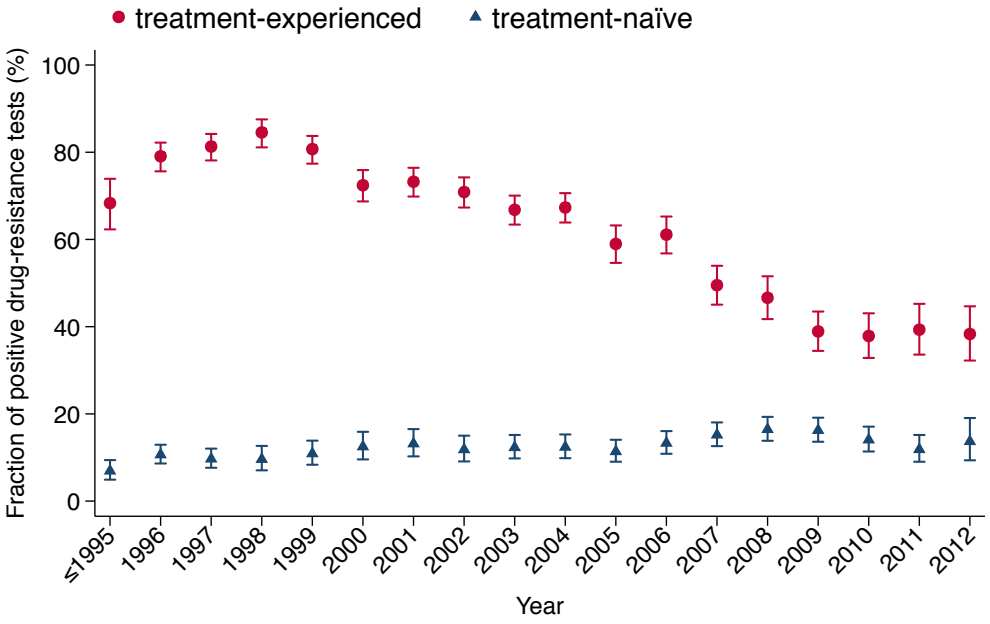
In the association analysis we applied Poisson regression to assess the association of TDR transmission with treatment-failing patients as potential transmitters. We considered annual rates of GRTs detecting TDR from recently-infected, treatment-naïve patients as outcome and PVL of all treatment-failing patients from the previous year as explanatory variable. We further studied the association for the most prevalent drug-resistance mutation for each major drug class in the SHCS: M184V, L90M, and K103N for NRTI, PI, and NNRTI, respectively. In this individual-mutation analysis, we fitted the model with the annual prevalence of each of these three transmitted mutations as outcome and the PVL of ART-failing patients carrying the corresponding mutation from the previous year as explanatory variable. We performed sensitivity analyses including PVL from the same year of GRTs performed or from two years before (see Supplementary Material).

We expressed our results with 95% CI and two-sided p-values, with $p < 0.05$ being statistically significant. Data analyses were performed with Stata 13.0 SE (StataCorp, Texas, USA).

Subgroup analysis

Considering that transmission to some SHCS patients may have occurred abroad and that the TDR prevalences of those patients would be less relevant to treatment-experienced patients in Switzerland, we repeated the association analyses with only those patients found in Swiss transmission clusters, defined phylogenetically [251]. To summarize, HIV-1 subtype B pol sequences from 8271 SHCS patients were pooled with foreign pol sequences from the Los Alamos Sequence database (n = 36230). Clusters were defined as clades

containing ≥ 10 sequences and consisting of $\geq 80\%$ sequences from the SHCS.



Year	≤ 95	1996	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012
experienced n=9616	262	616	663	517	622	620	721	690	798	771	524	532	503	414	468	362	285	248
naïve n=10504	535	837	744	459	524	449	479	491	610	598	651	692	705	736	721	605	456	212

Figure 8.1: Fraction of positive GRTs detecting any drug-resistance mutation for acquired and transmitted drug-resistance in the SHCS

20120 GRTs were generated in total before Jan.1, 2013 in the SHCS. 10504 GRTs (blue triangles) were performed from 7920 patients when they were treatment-naïve (regardless of recent infection), and 9616 GRTs (red dots) from 4816 individuals when they were treatment-experienced. Fractions of positive GRTs detecting any drug-resistance mutation for both populations were shown. The annual numbers of included GRTs from treatment-experienced (first row, red) and from treatment-naïve (second row, blue) patients were listed below the graph. Linear regression with fraction as dependent variable and year as explanatory variable showed that the fraction of positive drug-resistance tests in treatment-experienced patients has declined substantially over time (-2.8% per year [-3.4%, -2.2%]; $p < 0.001$), whereas the fraction of positive drug-resistance tests in treatment-naïve patients has not (0.3% per year [0.2%, 0.5%]; $p < 0.001$).
Vertical bars = 95% CI

8.3 Results

Fraction of positive GRTs in the SHCS

Figure 8.1 summarizes the fraction of TDR and ADR from all 20120 GRTs sampled before Jan. 1, 2013 regardless of the infection duration stratified by treatment status (naïve/experienced). Specifically, 10504 GRTs were from 7920 treatment-naïve and 9616 GRTs from 4816 treatment-experienced individuals.
ADR reached a peak at 85% in 1998 and dropped continuously since then to a plateau at 38% in 2009. This strong decrease of fraction of positive GRTs for ADR (linear regression: -2.8% / year [-3.4%, -2.2%]; $p < 0.001$) was not followed by a parallel decrease but rather a slight increase of fraction of positive GRTs for TDR (0.3% / year [0.2%,0.5%]; $p < 0.001$). To further dissect this discrepancy and to avoid possible bias introduced by

different persistence times of TDRs, we focused on studying treatment-naïve patients with GRTs performed within recent infection.

Study population including recently-infected, treatment-naïve and treatment-failing patients

We identified 2421 (31%) recently-infected patients from 7920 treatment-naïve patients in the SHCS with ≥ 1 GRT performed between June 26, 1992 and Dec.18, 2012. Additionally, we included 5399 patients having failed ≥ 1 regimen within years 1997 - 2011, presenting 18097 yearly-unique VL measurements. For detailed patient selection, see Figure 8.2. For representativeness of study population see Supplementary Figure S1.

TDR prevalences over time in recently-infected treatment-naïve patients and associated risk factors

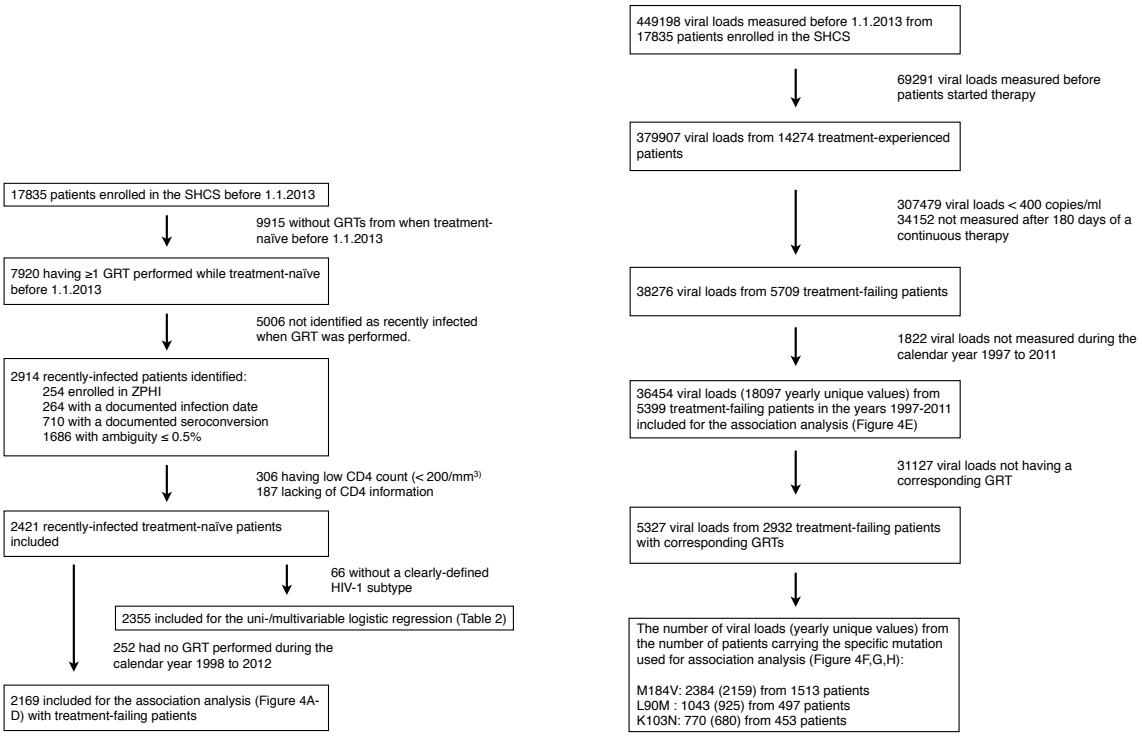
TDR prevalences fluctuated substantially over time with the median (range) as follows: 9.1% (2.2%, 15.6%) to any drug; 5.8% (2.2%, 14.3%) to NRTI; 2.5% (0, 4.8%) to PI; 1.4% (0, 5.1%) to NNRTI (Figure 8.3, black dots).
We observed two opposing developments in the multivariable logistic model that could explain the complex fluctuations of TDR prevalences (Table 8.1). On the one hand, overall TDR prevalence dropped after introduction of new drug classes. In particular, prevalences of TDRs significantly dropped after PI/r and InSTI became available. On the other hand, we found a linear increase of TDR prevalences when the number of available drug classes remained constant (Figure 8.3). The combination of these two opposing developments resulted in TDR prevalences, which increased in the absence of new drugs but decreased sharply upon introduction of new drug classes. TDR prevalences predicted from this model were shown in Figure 8.3 (blue lines). Additionally, prevalences of TDR to individual drug classes showed similar but not significant patterns as mentioned above (Supplementary Table S1.1-S1.3).

Association of drug-resistance transmission with the viral burden of treatment-failing patients

We further investigated whether drug-resistance transmission was associated with treatment-failing patients. We fitted annual prevalences of any TDR (outcome) and PVL of treatment-failing patients from the previous year (explanatory variable) with a Poisson regression model. The rate ratio (RR) was 0.91/1000 PVL (0.83, 0.99; $p = 0.033$), indicating a 9% increase of TDR prevalence for a decrease of PVL of ART-failing patients from the previous year by 1000 (Figure 8.4 A,E). PVL itself decreased over time (linear regression: -318/year [-438, -197]; $p < 0.001$). When we considered patients identified in Swiss transmission clusters, we found no discernible evidence for an association between TDR and PVL (RR=0.76/1000 PVL [0.43, 1.34]; $p = 0.34$).
Taken together, our results suggested no or a negative association between TDR prevalences and PVL of ART-failing patients from the previous year.

Transmission of the class specific drug-resistance mutations M184V, L90M, K103N

The above analysis pooled all TDRs and potentially neglected the differential behavior of individual mutations. We therefore performed individual-mutation analysis for the most prevalent drug-resistance mutation for each drug class.



(A) Recently-infected, treatment-naïve population

(B) Treatment-failing population

Figure 8.2: Selection profile for the recently-infected, treatment-naïve and the treatment-failing population

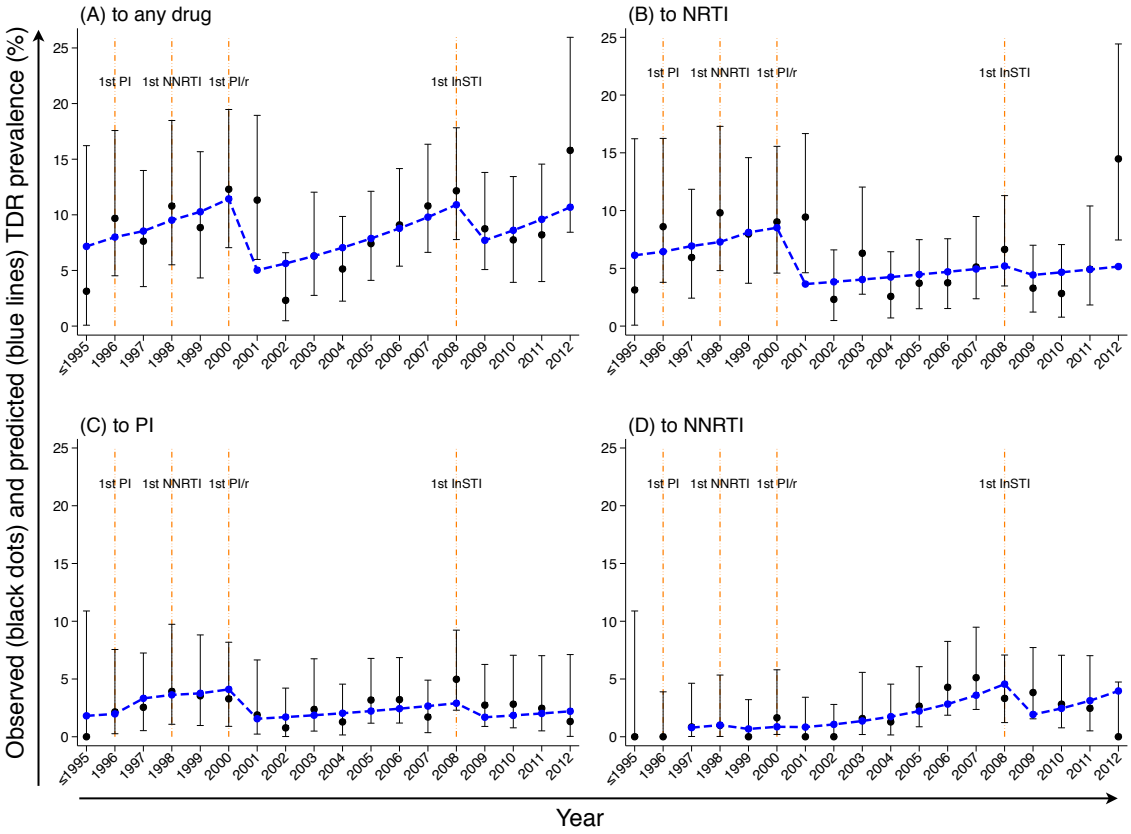
Numbers outside of the box indicate exclusions. (A) Selection profile for the recently-infected, treatment-naïve population. We selected patients enrolled in the SHCS before Jan.1, 2013 with GRTs performed when they were treatment-naïve (n=7920). From them we identified patients having GRTs performed during recent infection ($\leq$ 1 year of infection) according to documented infection dates, seroconversions, or ambiguity score and CD4 count. These patients thus constitute our basic study population (n=2421). For further analyses such as for the uni- and multivariable analysis in Table 8.1 and for the association analyses in Figure 8.4 A-D, 66 and 252 patients were excluded due to additional criteria set for these analyses. 66 patients did not have a clearly defined subtype, and 252 patients were not sampled between 1998 - 2012 (for details see individual descriptions in Table 8.1 and Figure 8.4).
(B) Selection profile for the treatment-failing population. We chose PVL as an indicator for the viral burden from treatment-failing patients on a population level. PVL was defined as the sum of $\log_{10}$ transformed VLs from treatment-failing patients. We thus selected available VL measurements from SHCS patients when they were treatment-experienced. High VLs ($\geq$ 400 copies/$m\ell$) measured after 180 days of and during a continuous therapy were included from these patients. Because VL has been fully integrated into clinical routine since 1997, values before 1997 were excluded. We calculated a yearly-unique VL from each patient (if $\geq$ 1 VL was available per patient within the same year, the mean was used) and used these values for association analysis in Figure 8.4 E. For further association analyses as in Figure 8.4 F-H, where we studied the transmission pattern of a specific TDR, only VLs corresponding with a GRT were included because genotyping was needed to determine drug-resistance mutations. From VLs having corresponding GRTs we selected those from patients carrying M184V, L90M, or K103N for association analysis in Figure 8.4 F, G, or H, respectively.

	No. with resistance / Total No. in subgroup (%)[a,b]	OR (95% CI) in univariable analysis	p-value	OR (95% CI) in multivariable analysis	p-value
Age	35 (28, 42)[c]	1.00 (0.98 - 1.01)	0.62		
Ethnicity			0.33		
Caucasian	182/1985 (9.2)	1.00 (Ref.)			
Black	16/222 (7.2)	0.77 (0.45 - 1.31)			
Others[c]	9/148 (6.1)	0.64 (0.32 - 1.28)			
HIV Subtype			<0.01		0.03
B	167/1683 (9.9)	1.00 (Ref.)		1.00 (Ref.)	
Non-B	40/672 (6.0)	0.57 (0.40 - 0.82)		0.65 (0.43 - 0.98)	
Sex			0.07		0.10
Male	173/1853 (9.3)	1.00 (Ref.)		1.00 (Ref.)	
Female	34/502 (6.8)	0.71 (0.48 - 1.03)		0.96 (0.60 - 1.55)	
Transmission Group[d]			0.03		0.62
MSM	129/1248 (10.3)	1.00 (Ref.)		1.00 (Ref.)	
HSX	52/770 (6.8)	0.63 (0.45 - 0.88)		0.83 (0.52 - 1.30)	
IDU	22/263 (8.4)	0.79 (0.49 - 1.27)		0.86 (0.51 - 1.45)	
Others	4/74 (5.4)	0.50 (0.18 - 1.38)		0.57 (0.20 - 1.60)	
No. of available drug classes[e]			0.77		0.06[f]
1 (NRTI)	10/125 (8.0)	0.97 (0.49 - 1.91)		2.99 (0.99 - 9.02)	
2 (NRTI,PI)	20/220 (9.1)	1.12 (0.68 - 1.84)		2.85 (1.19 - 6.83)	
3 (NRTI,PI,NNRTI)	25/235 (10.6)	1.33 (0.84 - 2.11)		2.75 (1.36 - 5.55)	
4 (NRTI,PI,NNRTI,PI/r)	103/1252 (8.2)	1.00 (Ref.)		1.00 (Ref.)	
5 (NRTI,PI,NNRTI,PI/r,InSTI)	49/523 (9.4)	1.15 (0.81 - 1.65)		0.61 (0.34 - 1.07)	
Year	2005 (2001, 2008)[b]	1.02 (0.98 – 1.05)	0.32	1.13 (1.03 – 1.23)[g]	0.01

a. Number of patients with any drug resistance from the recently-infected, treatment-naïve patients with a clearly defined subtype (n = 2355).
b. For age and year, median (IQR) was shown
c. Others includes Asian, Hispanic, others, and unknown
d. MSM: men having sex with men, HSX: heterosexual, IDU: intravenous drug users, Others: others and unknown
e. NRTI: nucleotide reverse transcriptase inhibitor; PI: protease inhibitor; NNRTI: non-nucleotide reverse transcriptase inhibitor; PI/r: boosted protease inhibitor; InSTI: integrase inhibitor
f. p-value was obtained from the test for trend.
g. increment is per year

Table 8.1: Univariable and multivariable analysis for the overall TDR prevalences

We used logistic regression to model the odds of being detected as carrying TDR. The dependent variable was included as a binary response indicating whether any TDR was detected. All co-variables were categorical except for age and year that were continuous variables. In the multivariable model, we included significant variables from a univariable model: HIV subtype and transmission group. Variables chosen a priori to be included regardless of univariable significance were sex, number of available drug classes, and calendar year. Missing data were list-wise deleted, resulting that $66/2421 = 2.7\%$ of patients were deleted due to missing subtype.



Year	≤ 95	1996	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012
observed, n=2421	35	96	121	104	120	129	110	136	131	160	194	193	176	186	185	146	122	77
predicted, n=2355	32	93	118	102	113	122	106	130	127	156	189	187	176	181	183	142	122	76

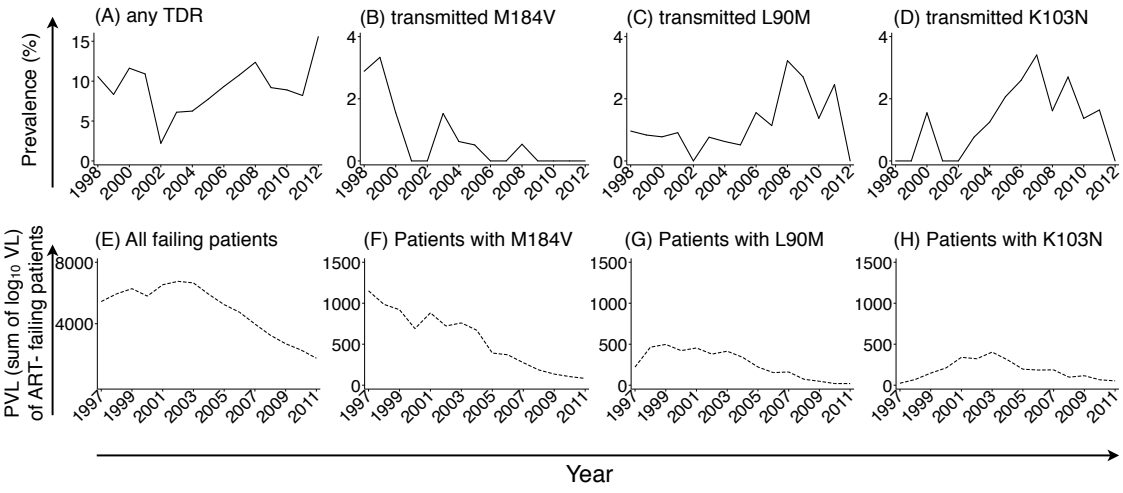
Figure 8.3: Observed and predicted TDR prevalences

2421 recently-infected, treatment-naïve patients with their first GRTs were included. For each year we calculated the percentage of GRTs detecting TDR (black dots) to (A) any drug, (B) NRTI, (C) PI, and (D) NNRTI. Additionally, we predicted TDR prevalence (blue dashed lines) by holding all co-variables except for year and number of available drug classes at baseline from the multivariable logistic regression model (Table 8.1) and transforming the odds obtained from the model. Co-variables included in the model were HIV-1 subtype and transmission group due to univariable significance and sex, number of available drug classes, and calendar year that were chosen a priori. Missing data were list-wise deleted. Total numbers of GRTs included for each year were listed at the bottom (for observed data in black; for predicted data in blue). The reason for a smaller sample size (n = 2355) for the predicted prevalences was that 66 patients were excluded from the multivariable model due to non-classified HIV-1 subtypes.
We found that the large fluctuations of the observed TDR prevalences (black dots) could be explained by two opposing developments: (1) a continuous increase with time when no new drug classes were introduced, and (2) a sharp decrease when a new drug class was introduced (orange vertical lines). This combined effect was described by the predicted TDR prevalence (blue dashed lines).
Vertical bars = 95% CI

Transmitted M184V increased 1.5 fold for an increase of PVL from ART-failing patients carrying M184V from the previous year by 100 (RR = 1.50/100 PVL [1.20, 1.86]; $p < 0.001$; Figure 8.4 B,F). This association increased to 6 fold when only TDRs from Swiss transmission clusters were considered (RR = 5.68/100 PVL [1.21, 26.7]; $p = 0.028$). On the contrary, we observed a negative association between the transmitted L90M and PVL from ART-failing patients carrying L90M from the previous year (RR = 0.75/100 PVL [0.58,0.96]; $p = 0.022$; Figure 8.4 C,G); the association became stronger when TDRs from Swiss transmission clusters were considered (RR=0.07/100 PVL [0.01, 0.46]; $p = 0.006$). For K103N no association was detected (RR = 1.00/100 PVL [0.73,1.37]; $p = 0.99$; Figure 8.4 D,H) and RR became negative when including only patients from Swiss transmission clusters but without reaching significance (RR = 0.02/100 PVL [0.0002, 1.55]; $p = 0.078$).
Sensitivity analyses using PVL from different years, and validating the ambiguity score for identifying recent infections showed that our results were robust (Supplementary Table S2, S3.1, S3.2). For a summary of sample size and method used in each analysis see Supplementary Table 4.

8.4 Discussion

In this study we investigated the paradox between the decrease in ADR prevalence [132, 230, 252] and a nearly stable prevalence of TDR [231, 233, 234, 248, 253]. If TDR indeed primarily originate from ART-failing patients with ADR, this discrepancy is counterintuitive. We therefore tested whether transmission of drug-resistant viruses was dependent on ART-failing patients in the SHCS, which is representative for Switzerland, over a 15-year time period. A large, clearly defined recently-infected, treatment-naïve population was used to calculate TDR prevalences.
Our results indicate that drug-resistance transmission is not predominantly driven by treatment-failing patients, but rather by a complex mixture of both ART-failing and ART-naïve patients. Despite PVL of treatment-failing patients decreased continuously, TDR prevalences increased over time. When specific TDRs were studied individually, distinct transmission patterns emerged. The prevalence of transmitted M184V correlated positively with PVL from ART-failing patients carrying M184V from the previous year. This association became stronger for patients included in Swiss transmission clusters. This suggests that the treatment-failing population is the major transmission source for M184V. In contrast, no positive association was found for L90M or K103N. We detected a negative association between prevalences of transmitted L90M and PVL from ART-failing patients carrying L90M from the previous year. This implies that major transmission reservoirs for these mutations are treatment-naïve rather than treatment-failing patients.
How can we explain such divergent transmission patterns between specific drug-resistance mutations? It is most likely due to the differential fitness costs, which represent the reduced ability of a virus harboring a drug-resistance mutation to replicate in the absence of the drug to which the mutation confers resistance. Generally, drug-resistant viruses will be replaced gradually by fitter viruses when drug pressure is not present, and the rate of the replacement depends on the degree of the fitness cost [146]. M184V disappears at a fast rate after transmission [214] without drug pressure due to its high fitness cost [254]. Therefore, M184V was rarely found in a drug-naïve population and its transmission depends on treatment-failing patients. In contrast, low-fitness-cost mutations L90M and K103N [178, 213, 255] persist longer in the absence of drug pressure [213], and may therefore persist within the ART-naïve population, which thus becomes an important source



Year	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012
TDR, n=2169	--	104	120	129	110	136	131	160	194	193	176	186	185	146	122	77
PVL(all failing patients), n=18097	1421	1528	1580	1437	1619	1631	1592	1411	1250	1149	986	813	668	570	442	--
PVL (failing patients with M184V), n=2159	292	253	236	179	233	185	195	176	100	94	74	52	38	30	22	--
PVL (failing patients with L90M), n=925	50	107	111	99	110	90	101	84	54	38	40	19	12	5	5	--
PVL (failing patients with K103N), n=680	6	15	33	51	82	77	103	76	49	47	48	28	32	18	15	--

Figure 8.4: Association analysis for TDR prevalences with PVL from ART-failing patients from the previous year

Poisson regression was used to test the association between TDR and PVL from ART-failing patients from the previous year. 2169 patients with recently-infected, treatment-naïve GRTs during years 1998-2012 were included as the outcome to account for annual prevalences of (A) any TDR, (B) transmitted M184V, (C) transmitted L90M, and (D) transmitted K103N. Included as an explanatory variable was (E) PVL of all ART-failing patients, PVL of ART-failing patients carrying (F) M184V, (G) L90M, and (H) K103N, respectively, during years 1997 - 2011. Total numbers of GRTs performed from recently-infected, treatment-naïve patients for each year were listed in the first row of the table at the bottom. Annual numbers of yearly-unique VLs for all failing patients, noted as PVL (all failing patients), and PVL (failing patients with a specific mutation) were listed in the second to forth row.
We found that PVL of all treatment-failing patients has decreased over time (E; linear regression: -318 per year [-438, -197]; $p < 0.001$). Annual prevalences for any TDR was negatively associated with PVL of treatment-failing patients from the previous year (A, E; RR = 0.91 for every 1000 PVL-all increment [0.83, 0.99]; $p = 0.033$). Prevalence of transmitted M184V was positively associated with PVL from ART-failing patients carrying M184V from the previous year (B, F; RR = 1.50 for every 100 PVL increment [1.20, 1.86]; $p < 0.001$). On the other hand, a negative association and no association was found for L90M (C, G; RR = 0.75 for per 100 PVL increment [0.58, 0.96]; $p = 0.022$) and K103N (D, H; RR = 1.00 for per 100 PVL increment [0.73, 1.37]; $p = 0.99$), respectively.

for transmission of these mutations.
This interpretation is further supported by the fact that occurrence of L90M among recently-infected, treatment-naïve patients has increased years after the PVL from ART-failing patients carrying L90M started to decrease (Figure 8.4), resulting in the negative association from the Poisson regression. A similar but weaker phenomenon was observed for K103N. Various combinatorial ART-regimens might contribute to differences between transmission patterns of L90M and K103N. Drugs selecting for L90M, mainly saquinavir and nelfinavir, have been almost unused in Switzerland for many years, indicating circulation of transmitted L90M within the treatment-naïve population. On the other hand, drugs selecting for K103N, such as efavirenz and nevirapine, are still in heavy use, implying that transmission of K103N is fueled both by treatment-failing and treatment-naïve patients.
Complemented by results from previous phylogenetic analyses [51, 256, 257], our study further illustrates that the treatment-naïve population is a major source for ongoing transmission of low-fitness-cost mutations. Early diagnosis and treatment of HIV-1-infected individuals is warranted to block the otherwise self-fueling mechanism of unrecognized TDR, which persist in this population due to low fitness costs.
In the SHCS TDR prevalences fluctuated considerably over time. We hypothesized that introductions of new drugs had an effect on these fluctuations, because new drugs improve control of viremia in treated patients. Indeed, after each introduction of a new drug class, a drop in TDR prevalences was observed: in 1997 after introduction of PI, 1999 after NNRTI, 2001 after PI/r, and 2009 after InSTI (Figure 8.3). Despite the universal and unlimited access to ART in Switzerland, TDR prevalences could not be reduced over an 18-year study period (Figure 8.3 A). Possibly, even more TDRs would have occurred without a constant influx of new therapy options. This highlights the importance of a drug pipeline that constantly delivers new medications.
There are several limitations to this study. Although our study was limited to a single country, we believe that our findings are generalizable to settings with similar HIV epidemics and treatment policies (for generalizability see Supplementary Material). In the correlation analyses we used measures of treatment-failing patients from the previous year because we assumed that treatment-failing patients could transmit drug-resistance approximately within one year before salvage treatment is fully active. Sensitivity analyses using PVL from the same year or two years before revealed similar results to the original model (Supplementary Table S2). Furthermore, the lack of positive associations from individual-mutation analyses of L90M/K103N does not causally prove that treatment-naïve individuals are the main source for the transmission. Though unlikely due to the well-studied transmission dynamics within the SHCS [251], we cannot exclude that patients carrying the transmitted L90M/K103N in our study population might all have been infected abroad and thus the ART-failing PVL as measured in the SHCS would not be relevant. However, the subgroup analysis including only patients from Swiss transmission clusters confirmed the same finding.

Conclusions

We demonstrated that transmission of antiretroviral drug-resistance is temporarily reduced by the introduction of new drug classes and driven both by treatment-failing and treatment-naïve patients. These findings suggest a continuous need for new drugs, early detection and early treatment of HIV-1 infection to successfully control the spread of TDR in the long term.

8.5 Supplementary Materials

1. Generalizability of our findings
2. Supplementary Table S1.1: Multivariable analysis for TDR prevalences against NRTI
3. Supplementary Table S1.2: Multivariable analysis for TDR prevalences against PI
4. Supplementary Table S1.3: Multivariable analysis for TDR prevalences against NNRTI
5. Supplementary Table S2: Sensitivity analyses for the Poisson regression models including PVL from the same year or from previous two years as the independent variable
6. Supplementary Table S3.1: Sensitivity analysis for the multivariable logistic regression model including additionally the identification by ambiguity score as a co-variable
7. Supplementary Table S3.2: Sensitivity analyses for the Poisson regression models including additionally the identification by ambiguity score as a co- variable
8. Supplementary Table 4: Summary of sample size and statistical method
9. Supplementary Figure S1: Representativeness of recently-infected, treatment- naive patients included in our study according to the estimated numbers of newly diagnosed patients from the Swiss Federal Office of Public Health (FOPH) from 1996 - 2012

1. Generalizability:

The analysis provided in our manuscript originates from Swiss data. The Swiss HIV epidemic resembles very much the epidemic in other resource-rich countries in Europe, North America and Australia. These regions have obtained access to the same drugs at approximately the same time periods since the first drug AZT was approved in 1987 (sometimes there was a delay of 0.5-1 year for some drugs in some countries). Also transmission groups are comparable: MSM and heterosexuals and IDUs are the major transmission groups. For this reason, one would expect that the epidemic of transmitted drug resistance in these regions follows similar mechanisms as in Switzerland. Of course health systems can differ, access to HIV care can vary considerably between countries, which may lead to potentially different treatment outcomes and different rates of resistance. The Swiss HIV epidemic is characterized by several aspects: (i) the Swiss Health System has a very high standard, (ii) everybody since the beginning of the epidemic had full access to care and to all approved drugs independent of social status (this is also valid for immigrants, even asylum seekers during the often very lengthy admission procedure), (iii) the SHCS is running since 1988 and has enrolled more than 19'000 people as per today. A roughly similar cohort study (based on the population of these countries) means that in the US a cohort study would need to exist of approx. 830000 people, in Germany of 190'000 and in the UK of 142'500 including biobanks to be comparable to the SHCS. (iv) the SHCS has an unbiased approach meaning that all HIV-1 infected people of different transmission groups are enrolled at the same treatment centers and therefore obtain the same treatment (MSM, HSX, IDU, men, women) by the same ID/HIV-specialists. Taken together, the quality of HIV-1 care is good to very good in Switzerland and due to access of care for all HIV-1 infected people the "selection pressure" of antiretroviral drugs on the population level is strong. Hence, we believe that findings of our study should be generalizable to other developed countries where data needed to perform such an analysis are not available in the same manner. Moreover, our findings provide a best-case scenario for settings with a similarly intensive drug use but poorer surveillance; especially the finding that curbing the spread of resistance requires even in Switzerland the continuous introduction of new drug classes is extremely relevant in this regard: Given scale-ups of ART in many resource limited settings with limited means of surveillance, the selection pressure for drug resistance may very soon reach similar dimensions as in Switzerland but the same means to counteract it is lacking. Thus the finding that drug resistance remains a problem requiring constant introductions of new drug classes despite the nearly optimal conditions of medical care and therapy monitoring in Switzerland provides an important warning of the long-term risks associated with HIV drug resistance in resource limited settings.

Table S1.1: Multivariable analysis for TDR prevalences against NRTI[a]

	No. with resistance / Total No. in subgroup (%)[b]	OR (95% CI) in univariable analysis	P-value	OR (95% CI) in multivariable analysis	P-value
Age	35 (28, 42)[b]	1.00 (0.98 - 1.01)	0.63		
Ethnicity			0.35		
Caucasian	117/1985 (5.9)	1.00 (Ref.)			
Black	11/222 (5.0)	0.83 (0.44 - 1.57)			
Others[c]	5/148 (3.4)	0.56 (0.22 - 1.39)			
HIV Subtype			<0.01		<0.01
B	115/1683 (6.8)	1.00 (Ref.)		1.00 (Ref.)	
Non-B	18/672 (2.7)	0.38 (0.23 - 0.62)		0.38 (0.22 - 0.67)	
Sex					
Male	108/1853 (5.8)	1.00 (Ref.)	0.46	1.00 (Ref.)	<0.01
Female	25/502 (5.0)	0.85 (0.54 - 1.32)		1.17 (0.66 - 2.06)	
Transmission Group[d]					
MSM	80/1248 (6.4)	1.00 (Ref.)	0.25	1.00 (Ref.)	0.85
HSX	34/770 (4.4)	0.67 (0.45 - 1.02)		0.89 (0.51 - 1.55)	
IDU	16/263 (6.1)	0.95 (0.54 - 1.65)		0.82 (0.44 - 1.53)	
Others	3/74 (4.1)	0.62 (0.19 - 2.00)		0.66 (0.20 - 2.21)	
No. of available drug classes[e]			0.12		0.31[f]
1 (NRTI)	9/125 (7.2)	1.54 (0.75 - 3.19)		2.38 (0.65 - 8.69)	
2 (NRTI,PI)	17/220 (7.7)	1.66 (0.95 - 2.91)		2.44 (0.87 - 6.89)	
3 (NRTI,PI,NNRTI)	20/235 (8.5)	1.85 (1.09 - 3.13)		2.60 (1.13 - 5.98)	
4 (NRTI,PI,NNRTI,PI/r)	60/1252 (4.8)	1.00 (Ref.)		1.00 (Ref.)	
5 (NRTI,PI,NNRTI,PI/r,InSTI)	27/523 (5.2)	1.08 (0.68 - 1.72)		0.80 (0.38 - 1.67)	
Year	2005 (2001, 2008)[b]	0.97 (0.93 - 1.01)	0.12	1.05 (0.94 - 1.18)[g]	0.36

a.n=2355. Included in the logistic regression as the dependent variable was the binary response indicating whether TDR to NRTI was detected. All co-variables were categorical except for age and year which were continuous variables. For consistency we included in the multivariable model the same co-variables as in the model for TDR overall in Table 1: HIV subtype, transmission group, sex, number of available drug classes, and calendar year.
b.For age and year, median (IQR) was shown
c.others includes Asian, Hispanic, others, and unknown
d.MSM: men having sex with men, HSX: heterosexual, IDU: intravenous drug users, Others: others and unknown
e.NRTI: nucleotide reverse transcriptase inhibitor; PI:protease inhibitor; NNRTI: non-nucleotide reverse transcriptase inhibitor; PI/r: boosted protease inhibitor; InSTI: integrase inhibitor
f.p-value was obtained from the test for trend.
g.increment is per year

Table S1.2: Multivariable analysis for TDR prevalences against PI[a]

	No. with resistance / Total No. in subgroup (%)[b]	OR (95% CI) in univariable analysis	P-value	OR (95% CI) in multivariable analysis[c]	P-value
Age	35 (28, 42)[b]	1.00 (0.98 - 1.02)	0.96		
Ethnicity			0.02		
Caucasian	59/1985 (3.0)	1.00 (Ref.)			
Black	2/222 (1.0)	0.30 (0.07 - 1.22)			
Others[d]	1/148 (0.7)	0.22 (0.03 - 1.61)			
HIV Subtype			0.28		0.54
B	48/1683 (2.9)	1.00 (Ref.)		1.00 (Ref.)	
Non-B	14/672 (2.1)	0.72 (0.40 - 1.32)		1.25 (0.62 - 2.54)	
Sex			0.01		0.69
Male	56/1853 (3.0)	1.00 (Ref.)		1.00 (Ref.)	
Female	6/502 (1.2)	0.39 (0.17 - 0.91)		0.72 (0.25 - 2.07)	
Transmission Group[e]			<0.01		0.02
MSM	47/1248 (3.8)	1.00 (Ref.)		1.00 (Ref.)	
HSX	12/770 (1.6)	0.40 (0.21 - 0.77)		0.40 (0.16 - 0.98)	
IDU	3/263 (1.1)	0.29 (0.09 - 0.95)		0.30 (0.08 - 0.96)	
Others	0/74 (0)	-		-	
No. of available drug classes[f]			0.84		0.41[g]
1 (NRTI)	2/125 (1.6)	0.62 (0.15 - 2.62)		2.01 (0.24 - 16.69)	
2 (NRTI,PI)	7/220 (3.2)	1.25 (0.55 - 2.88)		3.12 (0.68 - 14.24)	
3 (NRTI,PI,NNRTI)	8/235 (3.4)	1.34 (0.61 - 2.95)		2.96 (0.86 - 10.15)	
4 (NRTI,PI,NNRTI,PI/r)	32/1252 (2.6)	1.00 (Ref.)		1.00 (Ref.)	
5 (NRTI,PI,NNRTI,PI/r,InSTI)	13/523 (2.5)	0.97 (0.51 - 1.87)		0.53 (0.19 - 1.44)	
Year	2005 (2001, 2008)[b]	1.01 (0.96 - 1.07)	0.75	1.09 (0.93 - 1.29)[h]	0.26

a. n=2355. Included in the logistic regression as the dependent variable was the binary response indicating whether TDR to PI was detected. All co-variables were categorical except for age and year which were continuous variables. For consistency we included in the multivariable model the same co-variables as in the model for TDR overall in Table 1: HIV subtype, transmission group, sex, number of available drug classes, and calendar year.
b. For age and year, median (IQR) was shown
c. 74 patients were omitted from the multivariable model for TDR prevalence to PI as the subgroup "others" of transmission group had zero TDR event. n=2281
d. Others includes Asian, Hispanic, others, and unknown
e. MSM: men having sex with men, HSX: heterosexual, IDU: intravenous drug users, Others: others and unknown
f. NRTI: nucleotide reverse transcriptase inhibitor; PI: protease inhibitor; NNRTI: non-nucleotide reverse transcriptase inhibitor; PI/r: boosted protease inhibitor; InSTI: integrase inhibitor
g. p-value was obtained from the test for trend.
h. increment per year

Table S1.3: Multivariable analysis for TDR prevalences against NNRTI[a]

	No. with resistance / Total No. in subgroup (%)[b]	OR (95% CI) in univariable analysis	P-value	OR (95% CI) in multivariable analysis[c]	P-value
Age	35 (28, 42)[b]	1.00 (0.98 - 1.03)	0.87		
Ethnicity			0.58		
Caucasian	40/1985 (2.0)	1.00 (Ref.)			
Black	5/222 (2.3)	1.12 (0.44 - 2.87)			
Others[d]	5/148 (3.4)	1.70 (0.66 - 4.37)			
HIV Subtype			0.58		0.79
B	34/1683 (2.0)	1.00 (Ref.)		1.00 (Ref.)	
Non-B	16/672 (2.4)	1.18 (0.65 - 2.16)		1.10 (0.54 - 2.25)	
Sex			0.08		0.18
Male	44/1853 (2.4)	1.00 (Ref.)		1.00 (Ref.)	
Female	6/502 (1.2)	0.50 (0.21 - 1.17)		0.42 (0.16 - 1.10)	
Transmission Group[e]			0.83		0.58
MSM	28/1248 (2.2)	1.00 (Ref.)		1.00 (Ref.)	
HSX	17/770 (2.2)	0.98 (0.53 -1.81)		1.76 (0.80 - 3.87)	
IDU	4/263 (1.5)	0.67 (0.23 - 1.93)		1.46 (0.48 - 4.46)	
Others	1/74 (1.4)	0.60 (0.08 - 4.45)		0.91 (0.12 - 6.98)	
No. of available drug classes[f]			0.11		0.24[g]
1 (NRTI)	0/125 (0)	-		-	
2 (NRTI,PI)	2/220 (0.9)	0.35 (0.08 - 1.47)		2.54 (0.34 - 19.15)	
3 (NRTI,PI,NNRTI)	2/235 (0.9)	0.33 (0.08 - 1.37)		1.32 (0.22 - 7.92)	
4 (NRTI,PI,NNRTI,PI/r)	32/1252 (2.6)	1.00 (Ref.)		1.00 (Ref.)	
5 (NRTI,PI,NNRTI,PI/r,InSTI)	14/523 (2.7)	1.05 (0.55 - 1.98)		0.32 (0.12 - 0.87)	
Year	2005 (2001, 2008)[b]	1.13 (1.05 - 1.21)	<0.01	1.28 (1.08 - 1.52)[h]	<0.01

a. n=2355. Included in the logistic regression as the dependent variable was the binary response indicating whether TDR to NNRTI was detected. All co-variables were categorical except for age and year which were continuous variables. For consistency we included in the multivariable model the same co-variables as in the model for TDR overall in Table 1: HIV subtype, transmission group, sex, number of available drug classes, and calendar year.
b. For age and year, median (IQR) was shown
c. 125 patients were omitted from the multivariable model for TDR prevalence to NNRTI as the subgroup 5 of number of available drug classes had zero TDR event. n=2230
d. Others includes Asian, Hispanic, others, and unknown
e. MSM: men having sex with men, HSX: heterosexual, IDU: intravenous drug users, Others: others and unknown
f. NRTI: nucleotide reverse transcriptase inhibitor; PI:protease inhibitor; NNRTI: non-nucleotide reverse transcriptase inhibitor; PI/r: boosted protease inhibitor; InSTI: integrase inhibitor
g. p-value was obtained from the test for trend.
h. increment is per year

Table S2: Sensitivity analyses for the Poisson regression models including PVL from the same year or from previous two years as the independent variable[a]

dependent and independent variables included in the model	PVL from the previous year (original model)		PVL from the same year (sensitivity analysis)		PVL from two years before (sensitivity analysis)	
	OR (95% CI)	p-value	OR (95% CI)	p-value	OR (95% CI)	p-value
TDR prevalence with PVL of ART-failing patients	0.91 (0.83 - 0.99)	0.03	0.92 (0.85 - 0.99)	0.03	0.91 (0.82 -1.01)	0.09
Prevalence of transmitted M184V with PVL of ART-failing patients carrying M184V	1.50 (1.20 - 1.86)	<0.01	1.36 (1.15 - 1.61)	<0.01	1.60 (1.21 - 2.10)	<0.01
Prevalence of transmitted L90M with PVL of ART-failing patients carrying L90M	0.75 (0.58 - 0.96)	0.02	0.74 (0.58 - 0.96)	0.02	0.76 (0.59 -0.98)	0.04
Prevalence of transmitted K103N with PVL of ART-failing patients carrying K103N	1.00 (0.73 - 1.37)	0.99	0.93 (0.67 - 1.29)	0.67	1.21 (0.89 - 1.64)	0.24
Patients found in Swiss transmission clusters						
TDR prevalence with PVL of ART-failing patients	0.76 (0.43 - 1.33)	0.34	0.86 (0.52 - 1.43)	0.56	0.70 (0.38 - 1.33)	0.28
Prevalence of transmitted M184V with PVL of ART-failing patients carrying M184V	5.68 (1.21 - 26.7)	0.03	2.38 (0.79 -7.13)	0.12	13.6 (0.73 -254.5)	0.09
Prevalence of transmitted L90M with PVL of ART-failing patients carrying L90M	0.07 (0.01 -0.46)	<0.01	0.04 (0.004 - 0.35)	<0.01	0.07 (0.01 - 0.47)	<0.01
Prevalence of transmitted K103N with PVL of ART-failing patients carrying K103N	0.02 (0.0002-1.55)	0.08	0.30 (0.01 - 7.10)	0.46	0.86 (0.07 - 10.4)	0.90

a.As sensitivity analyses we replaced the dependent variable, PVL from the previous year, with PVL from the same year with GRT, and from two years before GRT. Same analyses were repeated for patients found in Swiss transmission clusters.

Table S3.1: Sensitivity analysis for the multivariable logistic regression model including additionally the identification by ambiguity score as a co-variable[a]

	Original model		Sensitivity analysis	
Variables[b]	**OR (95% CI) in multivariable analysis**	**p-value**	**OR (95% CI) in multivariable analysis**	**p-value**
HIV Subtype		0.03		0.03
B	1.00 (Ref.)		1.00 (Ref.)	
NonB	0.65 (0.43 - 0.98)		0.64 (0.43 - 0.97)	
Sex		0.10		0.10
Male	1.00 (Ref.)		1.00 (Ref.)	
Female	0.96 (0.60 - 1.55)		0.95 (0.59 - 1.52)	
Transmission Group[c]		0.62		0.62
MSM	1.00 (Ref.)		1.00 (Ref.)	
HSX	0.83 (0.52 - 1.30)		0.81 (0.51 - 1.28)	
IDU	0.86 (0.51 - 1.45)		0.87 (0.51 - 1.46)	
Others	0.57 (0.20 - 1.60)		0.57 (0.20 - 1.62)	
No. of available drug classes[d]		0.06[e]		0.07[e]
1 (NRTI)	2.99 (0.99 - 9.02)		3.00 (0.98 -9.15)	
2 (NRTI, PI)	2.85 (1.19 - 6.83)		3.04 (1.26 - 7.33)	
3 (NRTI,PI,NNRTI)	2.75 (1.36 - 5.55)		2.84 (1.40 - 5.79)	
4 (NRTI,PI,NNRTI,PI/r)	1.00 (Ref.)		1.00 (Ref.)	
5 (NRTI,PI,NNRTI,PI/r,InI)	0.61 (0.34 - 1.07)		0.66 (0.37 - 1.17)	
Year	1.13 (1.03 - 1.23)	0.01	1.13 (1.03 - 1.24)	0.01
Identification by ambiguity			1.22 (0.91 - 1.64)[f]	0.20

a.This is to test the validity of ambiguity score to identify recent infections. We included additionally a binary co-variable in the multivariable logistic regression model showing whether a patient was identified as being recently-infected by ambiguity score. Results from the sensitivity analysis were shown on the right side.
b.Only variables included in the multivariable model were listed.
c.MSM: men having sex with men, HSX: heterosexual, IDU: intravenous drug users, Others: others and unknown
d.NRTI: nucleotide reverse transcriptase inhibitor; PI: protease inhibitor; NNRTI: non-nucleotide reverse transcriptase inhibitor; PI/r: boosted protease inhibitor; InSTI: integrase inhibitor
e.p-values were obtained from the test for trend.
f.Increment is per year

Table S3.2: Sensitivity analyses for the Poisson regression models including additionally the identification by ambiguity score as a co-variable[a]

	Original model		Sensitivity analysis	
	OR (95% CI)	p-value	OR (95% CI)	p-value
TDR prevalence with PVL of ART-failing patients	0.91 (0.83 - 0.99)	0.03	0.87 (0.78 - 0.99)	0.03
Prevalence of transmitted M184V with PVL of ART-failing patients carrying M184V	1.50 (1.20 - 1.86)	<0.01	1.67 (1.18 - 2.37)	<0.01
Prevalence of transmitted L90M with PVL of ART-failing patients carrying L90M	0.75 (0.58 - 0.96)	0.02	0.83 (0.62 - 1.12)	0.23
Prevalence of transmitted K103N with PVL of ART-failing patients carrying K103N	1.00 (0.73 - 1.37)	0.99	1.03 (0.74 - 1.44)	0.85

a.This is to test the validity of ambiguity score to identify recent infections. We included additionally a binary co-variable in the multivariable logistic regression model showing the fraction of GRTs identified by ambiguity score. Results from the sensitivity analysis were shown on the right side.

Supplementary Table 4: Summary of sample size and statistical method

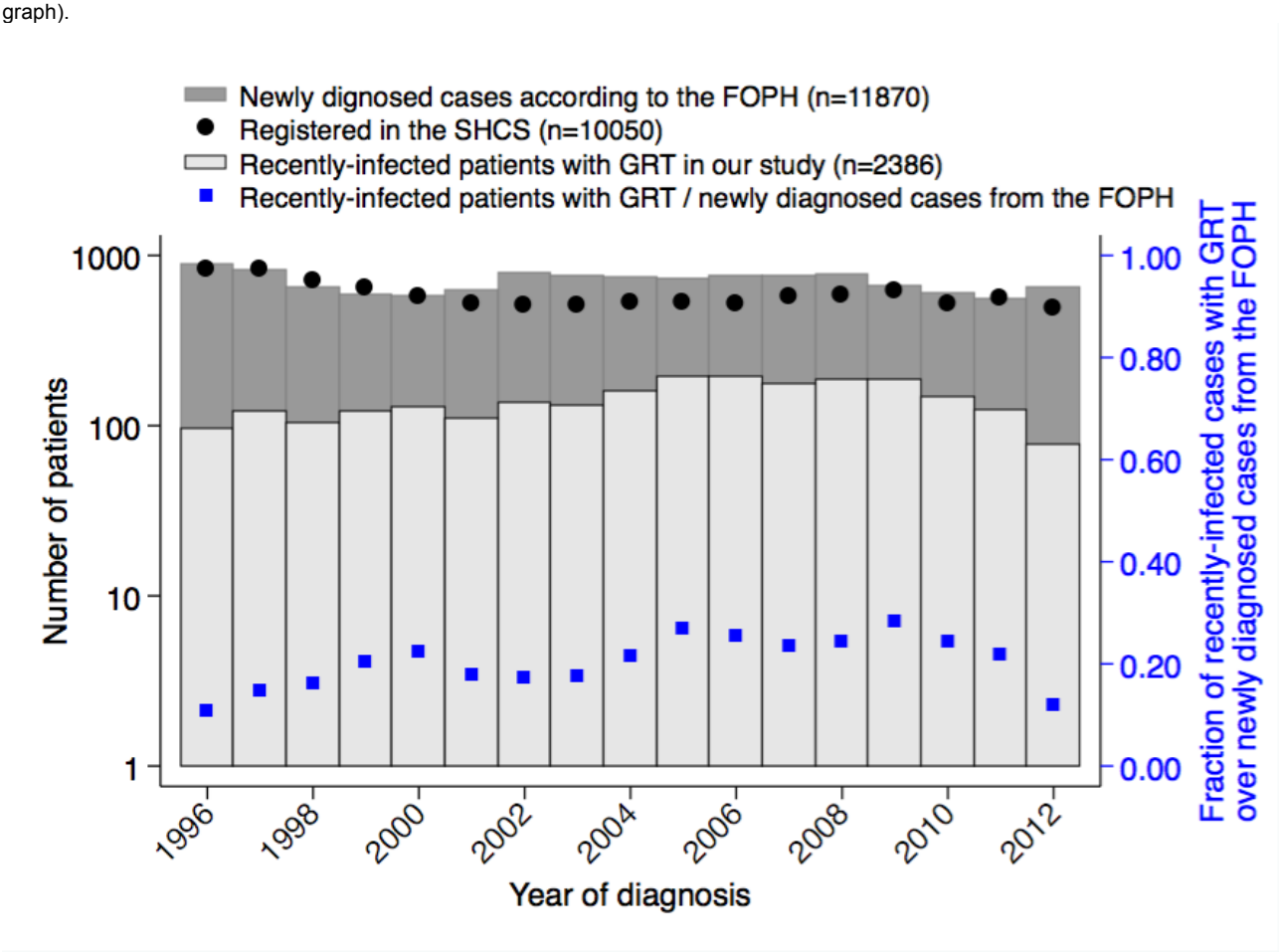
Analysis	Sample size	Method
Fraction of positive GRTs in Figure 1	• 10504 GRTs from 7920 treatment-naïve patients • 9616 GRTs from 4816 treatment-experienced patients	We calculated the annual percentages of GRTs detecting any drug-resistance mutations from patients of both groups
Prevalences of TDR in Figure 3	• 2421 recently-infected, treatment-naïve patients with their first GRTs	We calculated the annual percentages of GRTs detecting any drug-resistant, NRTI, PI, or NNRTI-resistant mutations
Uni-/multivariable analysis in Table 2 and prediction of TDR prevalences in Figure 3	• 2355 recently-infected, treatment-naïve patients	Model: logistic regression. Outcome: binary response of TDR detection Predictive prevalences were transformed from odds.
Association analysis for any drug-resistance mutations in Figure 4A, 4E	• 2169 recently-infected, treatment-naïve patients (during years 1998-2012) • 5327 VLs from 2932 treatment-failing patients (during years 1997-2011)	Model: Poisson regression. Outcome: TDR prevalences Explanatory: PVL from ART-failing patients
Association analysis for M184V in Figure 4B, 4F	• 2169 recently-infected, treatment-naïve patients (during years 1998-2012) • 2384 VLs (2159 yearly-unique VLs) from 1513 ART-failing patients	Model: Poisson regression. Outcome: prevalences of transmitted M184V Explanatory: PVL from ART-failing patients with M184V
Association analysis for L90M in Figure 4C, 4G	• 2169 recently-infected, treatment-naïve patients • 1043 VLs (925 yearly-unique VLs) from 497 ART-failing patients	Model: Poisson regression. Outcome: prevalences of transmitted L90M Explanatory: PVL from ART-failing patients with L90M
Association analysis for K103N in Figure 4D, 4H	• 2169 recently-infected, treatment-naïve patients • 770 VLs (680 yearly-unique VLs) from 453 ART-failing patients	Model: Poisson regression. Outcome: prevalences of transmitted K103N Explanatory: PVL from ART-failing patients with K103N

Analysis	Sample size	Method
Association analysis for any drug-resistance mutations for people in Swiss transmission clusters	• 632 recently-infected patients • 5168 VLs from 1490 ART-failing patients	Model: Poisson regression for patients in Swiss transmission clusters Outcome: TDR prevalences Explanatory: PVL from ART-failing patients
Association analysis for M184V for people found in Swiss transmission clusters	• 632 recently-infected patients • 598 VLs from 418 ART-failing patients	Model: Poisson regression for patients in Swiss transmission clusters Outcome: prevalences of transmitted M184V Explanatory: PVL from ART-failing patients with M184V
Association analysis for L90M for people found in Swiss transmission clusters	• 632 recently-infected patients • 213 VLs from 118 ART-failing patients	Model: Poisson regression for patients in Swiss transmission clusters Outcome: prevalences of transmitted L90M Explanatory: PVL from ART-failing patients with L90M
Association analysis for K103N for people found in Swiss transmission clusters	• 632 recently-infected patients • 148 VLs from 112 ART-failing patients	Model: Poisson regression for patients in Swiss transmission clusters Outcome: prevalences of transmitted K103N Explanatory: PVL from ART-failing patients with K103N

Supplementary Figures

Figure S1: Representativeness of recently-infected, treatment-naïve patients included in our study according to the estimated numbers of newly diagnosed patients from the Swiss Federal Office of Public Health (FOPH) from 1996 - 2012

According to the Swiss Federal Office of Public Health (FOPH), between 500 - 1000 people were estimated to be newly diagnosed each year from 1996 to 2012, resulting 11870 cases in total. This was in approximate accordance to the number of participants enrolled into the SHCS during the same time period (n=10050 from 1996-2012, dark gray bars and black dots, be aware of the log scale of this figure). Thus, for the time primarily studied in our paper, 1996-2012, the coverage of the SHCS is approximately 85% Among the newly diagnosed patients, 11% to 28% were included as being recently-infected in our study (blue squares) as defined in the method section. From 1996-2012 we included 2386 recently-infected, treatment-naïve patients (35 patients before year 1995 were not used for this graph).



9

Persistence of Transmitted HIV-1 Drug Resistance Mutations Associated with Fitness Costs and Viral Genetic Backgrounds

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Personal Contributions

For this study I identified all treatment-naïve patients carrying at least one transmitted resistance mutation and selected systematically all available longitudinal samples from these patients while being treatment-naïve for retrospective sequencing. After sequences were generated, I selected patients that matched our study criteria and summarized the baseline characteristics of the study population. I calculated the reversion rates of each drug resistance mutations using interval-censored survival models. I wrote the first version of the manuscript together with my co-shared first author and generated the Figure 9.1, Table 9.1, and the supplementary information.

Abstract

Transmission of drug-resistant pathogens presents an almost-universal challenge for fighting infectious diseases. Transmitted drug resistance mutations (TDRM) can persist in the absence of drugs for considerable time. It is generally believed that differential TDRM persistence is caused, at least partially, by variations in TDRM fitness costs. However, *in vivo* epidemiological evidence for the impact of fitness costs on TDRM-persistence is rare. Here, we studied the persistence of TDRM in HIV-1 using longitudinally-sampled nucleotide sequences from the Swiss HIV Cohort Study (SHCS). All treatment-naïve individuals with TDRM at baseline were included. Persistence of TDRM was quantified via reversion rates (RR) determined with interval-censored survival models. Fitness costs of TDRM were estimated in the genetic background in which they occurred using a previously published and validated machine-learning algorithm (based on *in vitro* replicative capacities) and were included in the survival models as explanatory variables.

In 857 sequential samples from 168 treatment-naïve patients, 17 TDRM were analyzed. RR varied substantially and ranged from 174.0/100 person years; 95% CI, [51.4, 588.8] (for 184V) to 2.7/100 person years; [0.7, 10.9] (for 215D). RR increased significantly with fitness cost (increase by 1.6 [1.3, 2.0] per standard deviation of fitness costs). When subdividing fitness costs into the average fitness cost of a given mutation and the deviation from the average fitness cost of a mutation in a given genetic background, we found that both components were significantly associated with reversion rates.

Our results show that the substantial variations of TDRM persistence in the absence of drugs are associated with fitness-cost differences both among mutations and among different genetic backgrounds for the same mutation.

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RESEARCH ARTICLE

Persistence of Transmitted HIV-1 Drug Resistance Mutations Associated with Fitness Costs and Viral Genetic Backgrounds

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Data Availability Statement: The baseline HIV-1 sequence data are accessible via GenBank. However, due to the sensitive nature of the data and concerns about compromising patient privacy from the reconstruction of transmission events, the longitudinally sampled sequences are not publicly available. All data in the Swiss-HIV-Cohort (http://www.shcs.ch) are available for well-defined projects according to the guidelines of the Swiss cohort if a corresponding project proposal is approved by the SHCS scientific board.

Abstract

Transmission of drug-resistant pathogens presents an almost-universal challenge for fighting infectious diseases. Transmitted drug resistance mutations (TDRM) can persist in the absence of drugs for considerable time. It is generally believed that differential TDRM-persistence is caused, at least partially, by variations in TDRM-fitness-costs. However, *in vivo* epidemiological evidence for the impact of fitness costs on TDRM-persistence is rare.

Here, we studied the persistence of TDRM in HIV-1 using longitudinally-sampled nucleotide sequences from the Swiss-HIV-Cohort-Study (SHCS). All treatment-naïve individuals with TDRM at baseline were included. Persistence of TDRM was quantified via reversion rates (RR) determined with interval-censored survival models. Fitness costs of TDRM were estimated in the genetic background in which they occurred using a previously published and validated machine-learning algorithm (based on *in vitro* replicative capacities) and were included in the survival models as explanatory variables.

In 857 sequential samples from 168 treatment-naïve patients, 17 TDRM were analyzed. RR varied substantially and ranged from 174.0/100-person-years;CI=[51.4, 588.8] (for 184V) to 2.7/100-person-years;[0.7, 10.9] (for 215D). RR increased significantly with fitness cost (increase by 1.6[1.3,2.0] per standard deviation of fitness costs). When subdividing fitness costs into the average fitness cost of a given mutation and the deviation from the average fitness cost of a mutation in a given genetic background, we found that both components were significantly associated with reversion-rates.

9.1 Introduction

Drug-resistant pathogens represent one of the major public health and clinical challenges in infectious diseases (`http://www.who.int/drugresistance/en/`). It is an almost universal observation that as soon as a chemotherapeutic agent against a given pathogen is introduced, resistant pathogen strains emerge, which reduce the clinical benefits conferred by that agent. One crucial obstacle in curbing drug resistance is that once it has emerged it often persists even in the absence of drug pressure. The central concept here is pathogen fitness: whereas the resistant pathogen has a very strong advantage over the sensitive one in the presence of drug pressure, its disadvantages in the absence of treatment are typically weaker and can be compensated by other mechanisms such as compensatory mutations or selection at linked loci. Despite this key role of pathogen fitness for a conceptual understanding of the spread and persistence of drug resistance, real-world epidemiological examples documenting its role are rare. An ideal opportunity to assess this role of fitness is provided by the dynamics of antiretroviral resistance in HIV-1.

In the case of HIV, combinations of modern anti-retroviral treatment (ART) have successfully reduced the morbidity and mortality of HIV-1 infected individuals [258]. Though drug resistance prevalence has been shown to decrease or to stabilize in various industrialized countries due to successful ART, it still remains a major concern jeopardizing treatment success [132, 259].

Transmission of a drug-resistant virus has been observed in most countries where ART is available [231, 243, 253, 260–263]. After transmission, viruses with transmitted drug resistance mutations (TDRM) persist either as the dominant species or as minority variants, which are difficult to detect by population sequencing techniques [213, 214, 220, 224, 244, 264, 265]. Consequently, patients harboring TDRM have a higher chance to fail their first-line therapy [218, 244, 266, 267].

Several studies have illustrated that the persistence time of individual TDRM in the absence of drug pressure exhibits substantial variance [178, 213, 214, 224, 265, 268]. Persistence times have been suggested to be associated with fitness costs [266], which are typically measured as the reduction of replicative capacity of the virus caused by a given mutation [178]. It is generally assumed that transmitted drug-resistant viruses revert more rapidly to wild-type viruses if the fitness is reduced to a larger extent by the TDRM (high fitness cost) because then reversion of TDRM confers correspondingly high fitness gains [146]. Several studies have measured the fitness of some specific TDRM using phenotypic replicative capacity assays [178, 260, 265]. However, evidence for the impact of such fitness costs on the dynamics of TDRM at an *in vivo* and epidemiological level is largely lacking. Here, we aimed to determine the persistence times of TDRM in an epidemiological approach *in vivo* and to determine whether these persistence times depend on the fitness costs of TDRM.

9.2 Methods

Study population

The SHCS is a prospective, nationwide, clinic-based study including a biobank. The SHCS is very representative of the HIV epidemiology in Switzerland; it includes at least 53% of all HIV cases ever diagnosed in Switzerland, 72% of all patients receiving ART, and 69% of the nationwide registered AIDS cases [10, 242]. Since 1996, the SHCS includes approximately 85% of the newly diagnosed HIV infected individuals in Switzerland. This number was obtained when we compared the estimated numbers of newly diagnosed HIV cases

published by the Swiss Federal Office of Public Health to the numbers of patients enrolled in the SHCS annually since 1996. Genotypic resistance data stem from routine clinical testing and from systematic retrospective sequencing before routine genotyping was introduced (over 11000 sequences were retrospectively generated). Genotyping is performed by four laboratories in Switzerland authorized by the Federal Office of Public Health. All laboratories perform population-based sequencing of the full protease gene and at least codons 28 - 225 of the reverse transcriptase gene using commercial assays such as Viroseq Vs.1 PE Biosystems; Virsoseq Vs. 2, Abbott AG; VircoTYPE HIV-1 Assay, Virco Lab or in-house methods [243] and has participated in the yearly quality control evaluation by the Agence Nationale de la Recherche du SIDA(ANRS) since 2002. All sequences are stored the SHCS drug resistance database using SmartGene's Integrated Dababase Network System (SmartGene, Zug, Switzerland, IDNS version 3.6.3) [244]. For details on the sequencing procedure, see [244]. To increase coverage, we have systematically selected all treatment-naïve individuals carrying TDRM and retrieved their sequential plasma samples before therapy from the SHCS biobank.
For this study we considered genotypic resistance test (GRT) performed for a patient when being treatment-naïve. All sequential GRTs were included for individuals having ≥ 2 GRTs and harboring TDRM at baseline before ever starting any antiretroviral therapy. TDRM was defined according to the WHO surveillance list of transmitted HIV drug resistance [224]. We studied mutations to the major three drug classes: nucleoside and nucleotide analogue reverse transcriptase inhibitors (NRTIs), protease inhibitors (PIs), and nonnucleoside reverse transcriptase inhibitors (NNRTIs). Additionally, we excluded 17 potential super-infections based on phylogenetic distance and the lack of phylogenetic clustering. Finally, since TDRM in HIV-1 CTL epitopes can disrupt binding to the HLA allele and such CTL-escape may essentially influence the reversion dynamics, we screened the list of optimal HIV-1 CTL epitopes (according to the Los Alamos HIV database, `http://www.hiv.lanl.gov/content/immunology/pdf/2013/optimal_ctl_article.pdf`) for epitopes containing TDRM and excluded from our analysis those mutations that disrupted binding to the epitope according to NetMHCcons (`http://www.cbs.dtu.dk/services/NetMHCcons/`).

Ethics statement

The SHCS, enrolling HIV-infected adults aged ≥ 16 years old, has been approved by ethics committees of all participating institutions. The data collection was anonymous and written informed consent was obtained from all participants [242].

Survival analysis

Our goal was to assess systematically the persistence of TDRM in the absence of drug pressure. In particular we considered the persistence across different mutations and viral genetic backgrounds (for a given mutation occurring in a given virus, the viral genetic background is given by the entire amino acid sequence in which this mutation is observed). To allow inter-patient comparisons we included TDRM that were present in at least five individuals at baseline.
We quantified the persistence via calculating reversion rates of individual TDRMs. Reversion of a TDRM was defined as an event at which a TDRM becomes undetectable by population sequencing assays. In other words, a TDRM has reversed when the HIV variant carrying that TDRM has decreased to the level below the detection limit of population sequencing assays ($\approx 20 - 30\%$ [269]). Therefore, reversion is not necessarily always to wild type. We fitted our data with an interval-censored survival model using exponential

waiting times. We chose an interval-censored model because the data did not allow us to determine the exact time point of reversion; instead a GRT not detecting a given resistance mutation preceded by a GRT with that mutation informs that the reversion event must have occurred in the time interval between those two tests.
Our results were expressed with 95% CI and two-sided p-values with $p < 005$ being statistically significant. We analyzed our data with Stata 13.1 SE (StataCorp, Texas, USA).

Estimation of fitness costs of TDRM

We estimated fitness costs based on a previously published approach to predict HIV replicative fitness from amino acid sequences [270]. This approach uses a machine-learning algorithm (ridge regression) trained on $>$ 70000 data points, each consisting of a *pol*-amino-acid sequence and an *in vitro* replicative capacity. Specifically, the algorithm predicts replicative capacity (pRC) from an amino acid sequence by a quadratic fitness model of the form

$$pRC(x) = \sum_{ij} M_{ij} x_i x_j$$

where x_i denotes the presence (1) or absence (0) of a given mutation i and M_{ij} the epistatic effects ($i < j$) and the main effects ($i = j$) characterizing the fitness landscape. These coefficients were derived in [270] by fitting the model to the $>$ 70000 data points. Since the number of parameters of the above model exceeds the number of data points, this model was fitted using an approach based on ridge regression. In essence, in this approach the data set was split into a training, training-test, and true-test data set. Then assuming a given penalty weight for model parameters, the model parameters are determined such that for the training data set, the sum of squared residuals plus the sum of squares of parameters times the penalty weight are minimized. In this specific case the approach was modified to a generalized linear ridge regression to take the non-normal error structure into account. The model was evaluated on the test-training data set, and the penalty weight was determined such that the predictive power on the test-training test was optimized. This final model was then evaluated on the true-test data set (which was used neither in deriving the model parameters nor in determining the penalty weight). Details on the method and validation on *in vitro* and clinical data can be found in [270] and [271].
Using this model, we estimated the fitness cost of a mutation in a given genetic background as follows. If A denotes the partial *pol*-amino-acid sequence (first 404 amino acid used in the reference [270]) with a given resistance mutation m and A' the same amino acid sequence but with the mutation reverted to its wild-type allele, then the fitness cost of the mutation m in the background A can be estimated as

$$c(m, A) = pRC(A)pRC(A').$$

A negative fitness cost was set to zero.
The impact of this fitness cost was assessed in univariable and multivariable versions of the interval-censored model. The multivariable models were adjusted for whether a given TDRM was present as a mixture with another amino acid at this position. Specifically, this was considered to be the case if the nucleotide sequence coding for this mutation contained at least one ambiguous nucleotide that affects the amino acid encoded.

Patients included	168
Age at baseline	35 (30.5, 40)
Gender	
Male	133 (79.2)
Female	35 (20.8)
Ethnicity	
White	147 (87.5)
Black	11 (6.5)
Others / Unknown	10 (6.0)
Transmission route	
MSM (Male Homosexual)	83 (49.4)
Heterosexual	47 (28.0)
Intravenous drug users	33 (19.6)
Unknown	5 (3.0)
Subtype	
B	137 (81.5)
Non-B	26 (15.5)
Non-classified	5 (3.0)
Viral load at baseline (log_{10} copies/ml) [a]	4.4 (3.6, 4.9)
CD4 count at baseline (cells/mm^c) [b]	494 (347, 656)
No. of mutations at baseline	
1	101 (60.1)
2	34 (20.2)
≥ 3	33 (19.6)
Mutations at baseline resistant to	
NRTI	101 (60.1)
PI	51 (30.4)
NNRTI	47 (28.0)
No. of resistant classes [c] at baseline	
1	142 (84.5)
2	21 (12.5)
3	5 (3.0)
Test interval in days	193 (170, 243)
Number of GRT performed	7 (4, 11)

a within 30 days before / after the first resistance test, N = 151 (90%)
b within 30 days before /after the first resistance test, N = 157 (93%)
c having ≥ 1 resistant mutations of a drug class

Table 9.1: Basic characteristics of study population

No. of patients (%) or Median (IQR) was shown.

9.3 Results

Study population

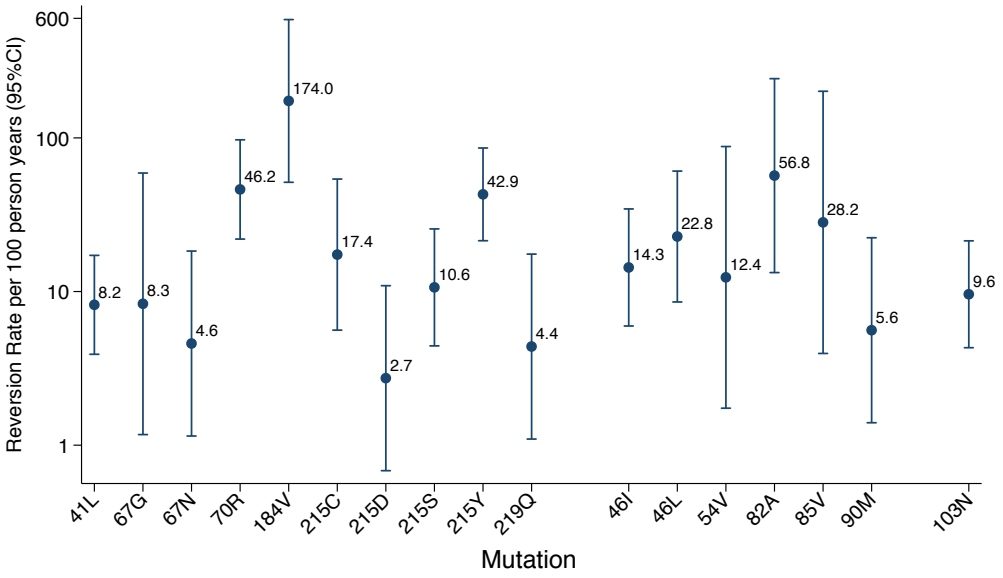
From 7920 treatment-naïve patients enrolled in the SHCS from May 1995 to February 2013, we could identify 987 sequential GRTs from 197 patients, who had ≥ 2 GRT while being treatment-naïve and presented with ≥ 1 TDRM at baseline. See S1 Table for all types and numbers of mutations and reversions observed from these 197 patients. The criterion that a given mutation must have been present in at least 5 individuals at baseline reduced the number of sequential GRTs and patients to 857 and 168, respectively.
From our studied population most individuals were male (80%), white (87.5%), and infected with subtype-B viruses (81.5%; Table 9.1). The median (IQR) number of GRT performed per person was 7 (4, 11) and the median (IQR) of test interval was 193 (170, 243) days. Baseline CD4 count was relatively high (494 [347, 656]), suggesting that patients were tested relatively early on after infection. 60.1% of patients had a single mutation detected at their first GRT. Detailed patient characteristics were shown in Table 9.1.

Reversion rate of individual TDRM varies

In total, 21 TDRM were analyzed. One mutation (190A of NNRTI) was excluded because we observed no reversion at all from the studied patients and three mutations (101E, 181C, 210W) were further excluded because they were located in the HLA epitopes (see Methods). Thus we could obtain reversion rates for 17 TDRM (Fig 9.1). Among them, 10 were mutations associated with resistance to NRTI, 6 to PI, and 1 to NNRTI. The quantified linear reversion rate showed that persistence time varied strongly among mutations. Among three drug classes, NRTI mutations showed the largest variability. Both the fastest and the slowest reversion rates, 174.0/100 person years [confidence interval = 51.4, 588.8] from 184V and 2.7/100 person years [0.7, 10.9] from 215D, respectively, belonged to this drug class.

Predicted fitness cost is associated with TDRM persistence

We found that reversion rates were associated significantly with the predicted fitness costs of resistance mutations (Fig 9.2). Specifically, the survival analysis with predicted fitness cost as an explanatory variable yielded that reversion rates increased by a factor 1.6 [1.3, 2.0] ($p < 0.001$) if fitness is increased by one standard deviation. Thus, predicted fitness has a considerable and highly significant impact on reversion rates. Since this analysis included different fitness costs of mutations, each in at least five patients, the observed effect of fitness can be caused by two mechanisms: On the one hand, by overall differences in costs among mutations (main effects) and, on the other hand, by different costs of the same mutation in different backgrounds (epistatic effects). In order to distinguish between these two effects, we further analyzed the data with two alternative approaches:
In the first approach, we still used predicted fitness cost as the explanatory variable but adjusted for the identity of the resistance mutation (i.e. the type of resistance mutation was included as a categorical variable). In this approach, the estimated effect of fitness corresponded to the impact of fitness within a given type of mutation. Since this approach introduced 17 variables for 264 data points and 62 events (and hence carries the risk of over-parameterization), we considered an alternative second approach, which only included two parameters. Specifically, we divided fitness cost into two components: the mean fitness cost of a mutation (across backgrounds) and the residual fitness cost, which is given as the difference between the predicted fitness cost in a given background and the mean



No. of reversion	7	1	2	7	4	3	2	5	8	2		5	4	1	2	1	2		6
Total at baseline	44	5	13	11	9	7	24	25	10	15		15	7	6	6	5	21		33
%	16	20	15	64	44	43	8	20	80	13		33	57	17	33	20	10		18

Figure 9.1: Reversion rate of individual TDRM

Reversion rate was quantified via an interval-censored survival model using an exponential distribution. The table below showed the number of reversion and total number observed at baseline for each TDRM. NRTI resistance mutations showed the largest variability that included both the fastest (184V) and the slowest (215D) reverting TDRM.

fitness cost. In the first approach, reversion rate was increased by a factor 1.8 [1.1, 3.1] ($p < 0.001$) if fitness cost was increased by one standard deviation (after adjusting for type of mutation). In the second approach, both mean fitness cost and residual fitness cost increased the reversion rate significantly by a factor 1.7 [1.3, 2.1] ($p < 0.001$) and 1.4 [1.1, 1.8] ($p = 0.007$) per standard deviation, respectively. Thus our models predict that a typical difference in fitness cost among resistance mutations (i.e. one standard deviation of the fitness costs observed in our data set), causes a 40% - 80% increase in the rate with which resistance mutations revert. Moreover, both approaches showed that both types of fitness cost (different overall costs of drug resistance mutations, and different costs in different backgrounds) are associated with higher reversion rates.
These multivariable models also showed that, as can be expected, reversion occurs much faster if a given TDRM is present as a mixture (see Methods, Table 9.2).

	Univar. HR (95% CI)	p	Multivar. HR (95% CI)	p
Mean fitness cost	1.31 (1.05,1.64)	0.015	1.65 (1.30,2.10)	<0.001
Residual fitness cost	1.34 (1.08,1.66)	0.008	1.38 (1.09,1.75)	0.007
TDRM present as mixture	9.71 (5.87,16.1)	<0.001	12.3 (7.22, 20.1)	<0.001

Table 9.2: Hazard ratios (HR) reported in univariable and multivariable models

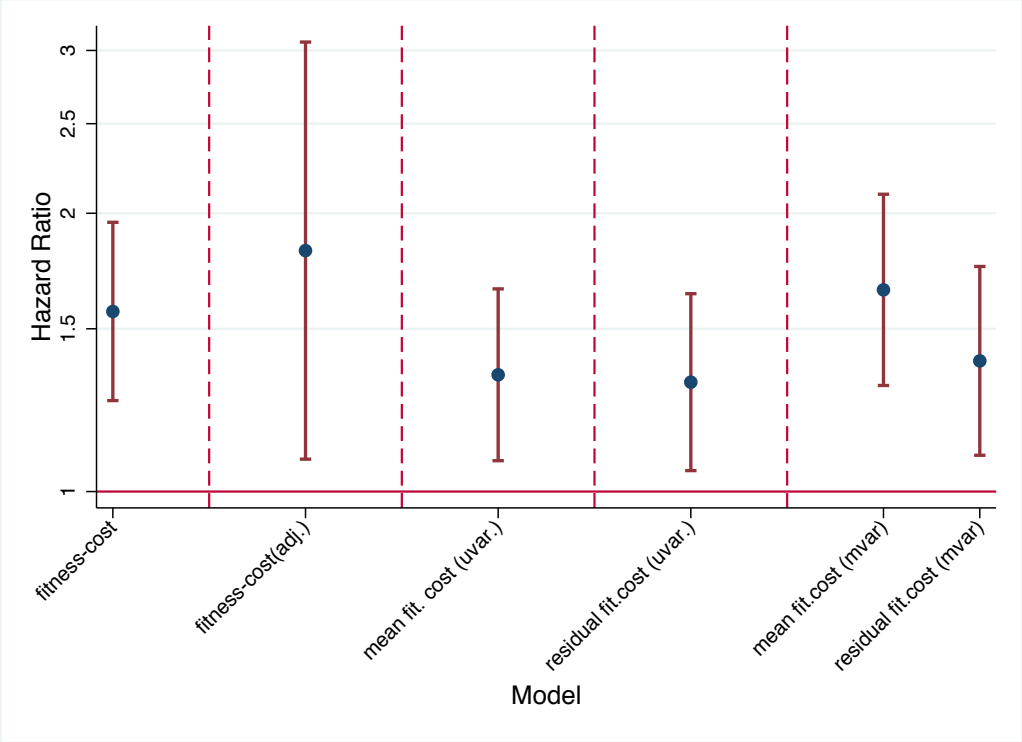


Figure 9.2: Impact of fitness cost on reversion rates

In unadjusted survival analysis (fitness cost), in survival analysis adjusted for type of mutation (fitness cost adj.). Impact of mean fitness cost and residual fitness cost in univariable analysis (uvar.) and in multivariable analysis including both mean and residual fitness cost (mvar.).

9.4 Discussion

In this study we investigated the differential persistence behaviors of TDRM in the absence of drug pressure and analyzed the association of the reversion rate with the predicted fitness cost of a given mutation. We used an interval-censored survival model to quantify the reversion rate of each mutation that was at least harbored by five individuals at baseline. We observed that the reversion rate of individual mutations varied substantially. Moreover, the reversion rates were significantly associated with the differential fitness costs of the TDRM: We showed that both the fitness-cost differences among mutations and among viral genetic backgrounds for the same mutation contributed to the variation in reversion rates. Thus, the novelty of this study is that we compared in total 17 TDRM from patients in a single cohort and could associate the persistence times with fitness costs of mutations predicted by a machine-learning model. An additional strength of this study is the high frequency and the number of resistance tests performed per patient.
Our results were consistent with most studies showing that M184V disappeared rapidly [178, 214, 215] whereas most thymidine analogue associated mutations (TAMs: 41L, 67N, 70R, 215Y, 219Q) disappeared at a slower rate [178, 215, 272] with the exception of 70R and 215Y. It is known, however, that 215Y has a high impact on fitness [178] and is rapidly replaced by intermediate 215S or atypical variants 215C/D [273]. Additionally, the fitness cost of 70R was shown to be higher when combined with other mutations *in vitro* [178, 242]. This could explain the observed high reversion rate of 70R regardless of its low fitness cost because in our data set 7 from 11 patients harboring 70R had at least one other mutation. Our data showed that most TDRM to PI reverted more rapidly,

compared to NRTI mutations.
From a more general perspective our findings have important implications for understanding the epidemic spread of drug-resistant pathogens. One of the general problems with drug resistance is that it can be quickly selected by drug pressure, but upon transmission it reverts only slowly if at all in the absence of drug pressure [274]. The intuition behind this is that drugs cause an enormous reduction in the replicative capacity of wild-type virus and hence lead to a strong relative fitness benefit for resistant mutants. By contrast, the fitness cost in the absence of drugs is typically weak. Our results highlight the large variability in reversion rates and the central role of fitness cost in governing the speed of reversion in the *in vivo* setting within the SHCS. In particular, they show that the genetic background of a resistance mutation substantially modulates the fitness cost and thereby the reversion rate of the mutation. This implies heritable variation in the fitness cost of resistance and thereby the danger that such fitness costs are reduced by evolutionary selection, i.e. mutations in genetic backgrounds causing lower fitness cost will have larger chances to spread to other patients and hence may dominate the population in the long run. Assessing the impact of the genetic background on reversion rates is central for understanding the spread of antimicrobial resistance in general. For example, theoretical models and *in vitro* evidence suggest a crucial role of compensatory mutations in boosting antibiotic resistance for a broad range of bacterial pathogens [275]. However, real-world epidemiological evidence for an impact of the genetic backgrounds found in natural pathogen populations on reversion of resistance in patients is largely lacking. In this context our approach offers a proof of principle for using machine learning approaches to bridge the gap between epidemiological data on resistance reversion and *in vitro* fitness measurements and thereby to address this crucial issue.
In the context of HIV epidemiology in Switzerland, such a scenario of mutation evolution can be probably prevented by the good surveillance and the early treatment of HIV-infected individuals, implying that resistant strains have only limited opportunity to cause new infections and hence to select backgrounds with lower fitness cost. By contrast, this scenario is a very real danger in settings with poorer surveillance and hence ampler opportunities for resistant viruses to spread. In those settings evolution might indeed successfully act on the variation of fitness costs and lead in the long term to resistant viruses with a low fitness cost.
Previous work [266] has assessed fitness costs of some antiretroviral resistance mutations *in vitro* by site directed mutations (SDM). Since these studies did not consider the impact of different genetic backgrounds, we can only compare the average fitness cost of a mutation determined by our method with the fitness costs determined by SDM. This comparison reveals a good qualitative but not perfect agreement to our estimates with SDM data (as summarized in [266]). Estimates were available in both data sets for the RT mutations 184V, 70R , 41L, 103N, and 215Y; in agreement with [266] we found a high fitness cost for 184V (1.8 standard deviations above mean fitness cost = +1.8s.d) and a moderate fitness cost for 70R , 41L, and 103N (+0.58 s.d. ,-0.16 s.d., and +0.48 s.d., respectively). In agreement with [266] we also found moderate fitness costs for 210W and 181C (-0.85 s.d. and -0.69 s.d. respectively), which were excluded from our analysis because they lie in HLA epitopes and disrupt binding. The main discrepancy was found for 215Y, where our methods predicted low fitness costs (- 0.86 s.d.) in contrast to the SDM data [266]. The fact that reversion rates are high for this mutation indicates that our estimator has underestimated the real fitness cost of this mutation. This failure may be also related to the complexity of the mutational pathways at this position, which may have been oversimplified by our approach (in which we do not distinguish which amino acid a TDRM reverts to). This deviation is also not surprising since the computational

predictor underlying our approach is not perfect (42% of deviance in *in vitro* fitness were explained in [270]). Overall this comparison thus validates our method but also reveals that there is potential for improvement and hence our approach should be best viewed as a proof of principle of using machine-learning approaches in conjunction with *in vitro* fitness measurements to assess reversion of TDRM *in vivo*.

This assessment of the fitness predictor is confirmed by considering the quality of fit of the different models summarized in Fig 9.2: Starting from an interval-censored survival model without explanatory variables, adding the information of whether a given TDRM is present as a mixture reduces the model deviance by 22%. Adding TDRM-fitness as an explanatory variable reduces the model's deviance by a further 9%. If we separate fitness cost into the mean fitness cost of a given mutation type and the corresponding residual fitness cost (as in Fig 9.2), this 9% results from a 6% of deviance-reduction explained by the mean fitness cost and 3% by the residual fitness cost. This indicates an important role of fitness for TDRM reversion; especially given that, firstly, the fitness predictor used here is not perfect (it explains 42% of deviance of *in vitro* replicative capacity [270]) and that, secondly, being a mixture implies that a nucleotide has already started to revert and hence the corresponding variable represents a very strong determinant of reversion. Finally, these numbers suggest that the differential fitness-costs of the same mutation in different genetic backgrounds contribute half as much to the population-level variability in reversion than different fitness-costs of different mutations. Given the well-described and strong differences in reversion rates across mutation types this therefore implies an important role of the genetic background. However, these fractions of deviance explained by our predicted fitness costs imply that reversion rates also depend on other factors not captured by *in vitro* replicative capacity. This includes interactions between host-viral factors such as HLA escape. Even though we excluded TDRMs known to mediate CTL escape (see Methods), it is likely that this does not encompass all such escape mutations or more generally all mutations that affect the interaction of a virus with a given patients immune system.

Our study had several limitations. One of the limitations of this study was the lack of information before the first GRT was performed. More specifically, we could not determine how long a TDRM had already persisted before the first GRT. We studied the reversion of TDRM from the baseline GRT instead of the infection date of a patient because an exact infection date was not known for most of the patients and because GRTs at infection time are typically not available. This approach increased the sample size considerably in exchange for missing some TDRM that had reverted before the first GRT was performed. This could explain why K65R or T215F, which are known to revert rapidly, were not identified in our study. The fast reverting TDRM such as M184V were either missed or detected right after the infection by GRT, thus the estimated reversion rates were not altered to a large extent and only the sample size may be lower. Another limitation was that around 40% (67 / 168) of patients carried > 1 TDRM at baseline. Although combinations of mutations could modulate the fitness costs substantially [178], causing that a given mutation has varying fitness costs when having different genetic backgrounds, the number of mutations detected at the first GRT was not found to be associated with the reversion of TDRM [215]. Additionally we adjusted for different genetic backgrounds including the residual fitness costs in our model and still found positive associations of reversion rates with average fitness costs.

In conclusion, our study demonstrated that TDRM showed substantial variation in reversion rates, which were positively associated with the fitness costs these mutations had in their genetic background.

9.5 Supplementary

1. S1 Table

S1 Table. Observed frequency at baseline and number of reversion from mutations ever observed

Drug Class	Mutation	Number of reversion / Observed frequency at baseline (percentage)
NRTI	41L	9 / 45 (20.0)
	65R	2 / 3 (66.7)
	67G	1 / 5 (20.0)
	67N	3 / 14 (21.4)
	69D	1 / 1 (100.0)
	70E	1 / 1 (100.0)
	70R	7 / 11 (63.6)
	74I	1 / 1 (100.0)
	74V	1 / 3 (33.3)
	75A	1 / 1 (100.0)
	77L	0 / 1 (0.0)
	116Y	1 / 2 (50.0)
	151M	0 / 1 (0.0)
	184I	1 / 1 (100.0)
	184V	5 / 9 (55.6)
	210W	4 / 14 (28.6)
	215C	6 / 8 (75.0)
	215D	4 / 24 (16.7)
	215E	1 / 3 (33.3)
	215F	1 / 4 (25.0)
	215I	2 / 1 (200.0)
	215S	8 / 25 (32.0)
	215V	0 / 1 (0.0)
	215Y	8 / 10 (80.0)
	219E	2 / 3 (66.7)
	219Q	2 / 15 (13.3)
	219R	3 / 4 (75.0)

Drug Class	Mutation	Number of reversion / Observed frequency at baseline (percentage)
PI	23I	1 / 1 (100.0)
	24I	0 / 2 (0.0)
	30N	1 / 2 (50.0)
	46I	5 / 16 (31.2)
	46L	5 / 8 (62.5)
	47V	3 / 3 (100.0)
	48V	0 / 1 (0.0)
	50V	1 / 1 (100.0)
	53L	0 / 1 (0.0)
	54M	0 / 1 (0.0)
	54V	1 / 6 (16.7)
	73C	1 / 2 (50.0)
	73S	2 / 2 (100.0)
	76V	1 / 1 (100.0)
	82A	2 / 6 (33.3)
	82L	0 / 1 (0.0)
	82T	1 / 1 (100.0)
	83D	0 / 1 (0.0)
	84V	1 / 3 (33.3)
	85V	1 / 5 (20.0)
	88D	1 / 2 (50.0)
	90M	2 / 21 (9.5)

Drug Class	Mutation	Number of reversion / Observed frequency at baseline (percentage)
NNRTI	101E	2 / 5 (40.0)
	101P	0 / 1 (0.0)
	103N	9 / 35 (25.7)
	103S	0 / 2 (0.0)
	179F	1 / 1 (100.0)
	181C	2 / 8 (25.0)
	188L	1 / 3 (33.3)
	190A	0 / 7 (0.0)
	225H	0 / 1 (0.0)

10

Assessing efficacy of different nucleos(t)ide backbones in non-nucleoside reverse transcription inhibitor containing regimens in the Swiss HIV Cohort Study

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Personal Contributions

Part of the genotypic resistance tests originated from my systematic selection of samples for retrospective sequencing (for details see Chapter 7). I searched and manually corrected the SHCS database for patients' tablet usage of antiretroviral treatment. I designed all statistical models, performed all analyses, and generated all figures and tables. Moreover, I wrote the first version of the manuscript and edited it after comments from the co-authors.

Abstract

Background
The most recommended NRTI combinations as first-line antiretroviral treatment for HIV-1 infection in resource rich settings are tenofovir(TDF)/emtricitabine(FTC), abacavir(ABC)/lamivudine(3TC), TDF/3TC and zidovudine(AZT)/3TC . Different pill numbers and dosing frequencies per day among these combinations were rarely considered when studying their relative efficacy.
Methods
We included patients starting their first-line cART with or switching from the first-line cART without treatment failure to TDF/FTC, ABC/3TC, TDF/3TC, or AZT/3TC plus efavirenz or nevirapine. Cox proportional hazard regression was used to investigate the effect of the different NRTI combinations on two primary outcomes: virological failure (VF) and emergence of NRTI-resistance. Additionally, we performed a pill burden analysis and adjusted the model for pill number and dosing frequency.
Results
Compared to TDF/FTC, ABC/3TC had an adjusted hazard ratios (HR) for having VF of 2.01 (95% CI, 0.86 - 4.55), TDF/3TC 2.89 (1.22 - 6.88), and AZT/3TC 2.28 (1.01 - 5.14), whereas for the emergence of NRTI resistance ABC/3TC had a HR of 1.17 (0.11 - 12.2), TDF/3TC 11.3 (2.34 - 55.3), and AZT/3TC 4.02 (0.78 - 20.7). Differences among regimens disappeared when models were additionally adjusted for pill burden. However, non-white population compared to white population and higher pill number per day were associated with increased risks of VF and emergence of NRTI-resistance: HR of ethnicity for VF was 2.85 (1.64 - 4.96), for NRTI resistance 3.54 (1.20 - 10.4); HR of pill burden for VF was 1.41 (1.01 - 1.96), for NRTI resistance 1.72 (0.97 - 3.02).
Conclusions
Pill burden and ethnicity showed a robust association with virological response and emergence of NRTI resistance.

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Assessing efficacy of different nucleos(t)ide backbones in NNRTI-containing regimens in the Swiss HIV Cohort Study

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Background: The most recommended NRTI combinations as first-line antiretroviral treatment for HIV-1 infection in resource-rich settings are tenofovir/emtricitabine, abacavir/lamivudine, tenofovir/lamivudine and zidovudine/lamivudine. Efficacy studies of these combinations also considering pill numbers, dosing frequencies and ethnicities are rare.

Methods: We included patients starting first-line combination ART (cART) with or switching from first-line cART without treatment failure to tenofovir/emtricitabine, abacavir/lamivudine, tenofovir/lamivudine and zidovudine/lamivudine plus efavirenz or nevirapine. Cox proportional hazards regression was used to investigate the effect of the different NRTI combinations on two primary outcomes: virological failure (VF) and emergence of NRTI resistance. Additionally, we performed a pill burden analysis and adjusted the model for pill number and dosing frequency.

Results: Failure events per treated patient for the four NRTI combinations were as follows: 19/1858 (tenofovir/emtricitabine), 9/387 (abacavir/lamivudine), 11/344 (tenofovir/lamivudine) and 45/1244 (zidovudine/lamivudine). Compared with tenofovir/emtricitabine, abacavir/lamivudine had an adjusted HR for having VF of 2.01 (95% CI 0.86–4.55), tenofovir/lamivudine 2.89 (1.22–6.88) and zidovudine/lamivudine 2.28 (1.01–5.14), whereas for the emergence of NRTI resistance abacavir/lamivudine had an HR of 1.17 (0.11–12.2), tenofovir/lamivudine 11.3 (2.34–55.3) and zidovudine/lamivudine 4.02 (0.78–20.7). Differences among regimens disappeared when models were additionally adjusted for pill burden. However, non-white patients compared with white patients and higher pill number per day were associated with increased risks of VF and emergence of NRTI resistance: HR of non-white ethnicity for VF was 2.85 (1.64–4.96) and for NRTI resistance 3.54 (1.20–10.4); HR of pill burden for VF was 1.41 (1.01–1.96) and for NRTI resistance 1.72 (0.97–3.02).

Conclusions: Although VF and emergence of resistance was very low in the population studied, tenofovir/emtricitabine appears to be superior to abacavir/lamivudine, tenofovir/lamivudine and zidovudine/lamivudine. However, it is unclear whether these differences are due to the substances as such or to an association of tenofovir/emtricitabine regimens with lower pill burden.

Introduction

More than 25 antiretroviral drugs from 6 different drug classes against HIV-1 infection are available today. The standard combination ART (cART) consists of two NRTIs and a potent third agent, e.g. an NNRTI.[1] Recent guidelines recommend tenofovir/emtricitabine or abacavir/lamivudine in combination with either efavirenz or nevirapine, or rilpivirine for individuals with

10.1 Introduction

More than 25 antiretroviral drugs from 6 different drug classes against HIV-1 infection are available today. The standard combination of antiretroviral treatment (cART) consists of two nucleos(t)ide reverse transcriptase inhibitors (NRTIs) and a potent third agent, generally a non-NRTI (NNRTI) or a boosted protease inhibitor (PI/r) or more recently an integrase inhibitor [53]. For first-line cART including NNRTI, recent treatment guidelines recommend tenofovir (TDF)/emtricitabine (FTC) or abacavir (ABC)/lamivudine (3TC) as the preferred NRTI backbone in combination with either efavirenz (EFV) or nevirapine (NVP), or rilpivirine for individuals with HIV-1-RNA <100000 copies/$m\ell$ [53, 130]. Alternatively, if unavailability or intolerance to other recommended NRTIs exists, TDF/3TC and zidovudine (AZT)/3TC are recommended [276, 277]. These were widely used in first-line regimens before the availability of TDF/FTC, ABC/3TC as fixed dose combinations in resource rich countries and are still widely used in resource limited settings.
Studies directly comparing all these four important NRTI combinations in large populations are lacking. The relative in vivo efficacy of these recommended NRTI combinations is unclear. AZT/3TC was shown to have similarly high potency as TDF/FTC containing EFV in a randomized controlled trial [278] but is rarely the first option nowadays for treatment in resource rich settings due to toxicity [279], intolerability issues and twice daily dosing [136]. Although ABC/3TC and TDF/FTC were found in a randomized trial to provide comparable antiretroviral efficacy for first-line treatment [280], in another clinical trial ABC/3TC showed inferior virological responses than TDF/FTC in patients with baseline HIV-RNA levels > 100000 copies/$m\ell$ [129, 281]. ABC/3TC was also associated with more adverse events including lipid abnormalities [281]. Moreover, some randomized trials observed better virological responses for regimens containing TDF/FTC than TDF/3TC [282] whereas other studies [283, 284] observed equal suppression rates between the two. On the other hand, a recent observational study comparing treatment-naïve patients initiating TDF/3TC or TDF/FTC plus a NNRTI found that TDF/3TC led to more virological failures, however this study did not consider adherence, pill counts, or dosing frequency as potential confounders [285].
Comparing NRTI backbones is a complex undertaking because they are formulated differentially: for TDF/FTC, ABC/3TC once-daily and for AZT/3TC twice-daily fixed dose combinations (FDC) exist. EFV can be given in combination with FDC but mostly is used as single tablet regimen including EFV/TDF/FTC. In addition, 3TC can be taken once or twice daily in contrast to FTC, which has exclusively the once-daily option. Thus, the daily number of total pills and the maximal dosing frequency can vary substantially among AZT, ABC, FTC, 3TC, and TDF in NNRTI containing regimens. Randomized clinical trials mostly compare just two, if at all, backbones against each other. In addition, they do not necessarily reflect a routine clinical setting because often patients are highly selected due to strict enrolment criteria and men are enrolled over proportionally in general. However, it is of high importance to examine the treatment efficacy of NRTI backbones with regards to pill burden and dosing frequency, since governments, health insurances and third-party payers may soon start to put pressure on using also non-coformulated ART generics in the future due to considerably lower prices.
Therefore, the aim of this study was to compare TDF/FTC, ABC/3TC, TDF/3TC and AZT/3TC paired with EFV or NVP as first-line cART regarding virological responses and emergence of drug-resistance in the representative Swiss HIV Cohort study (SHCS) and to evaluate the impact of pill burden and dosing frequency on treatment efficacy.

10.2 Methods

Selection of patients

Our analysis was based on ART-experienced patients from the Swiss HIV Cohort Study starting treatment up to January 8, 2014. The SHCS, continually enrolling patients aged 16 or older since 1988, is a prospective and nationwide cohort study including a biobank. The SHCS is representative for the HIV epidemic in Switzerland; it includes at least 53% of all HIV cases ever diagnosed in Switzerland, 72% of all patients receiving ART, and 69% of the nationwide registered AIDS cases [242]. Ethical committees of all participating institutions have approved the SHCS, and written informed consent was obtained from all patients [10, 242].
Resistance data are generated from routine-clinical testing performed by four laboratories, which were authorized by the Swiss Federal Office of Public Health. All laboratories sequenced the protease and the reverse transcriptase gene using population-based sequencing with commercial assays (Viroseq Vs.1 PE Biosystems; Viroseq Vs. 2, Abbott AG; VircoTYPE HIV-1 Assay, Virco Lab) or in-house methods [286]. They all participate in the annual quality control evaluation by the Agence Nationale de la Recherche sur le SIDA et les hpatites virales (ANRS) since 2002. All sequences are entered into the SHCS drug resistance database using SmartGene's Integrated Dababase Network System (SmartGene, Zug, Switzerland, IDNS version 3.6.3) [244]. Additionally, we systematically selected and retrospectively sequenced plasma samples from drug naïve and for treatment failing patients stored in our biobank, especially for samples obtained before the routine genotyping was introduced.
To compare the efficacy of the different NRTI backbones (TDF/FTC, ABC/FTC, ABC/3TC and AZT/3TC) either combined with EFV or with NVP, we identified HIV-1 infected patients from the SHCS who have initiated their first cART with one of the regimens mentioned above or switched from their first cART to one of these regimens for reasons other than treatment failure. Patients were excluded from the analysis if baseline resistance was identified according to the Stanford database algorithm (mutation penalty score ≥ 15, Stanford genotypic resistance interpretation algorithm version 7.0: `http://sierra2.stanford.edu/sierra/servlet/JSierra`). Patients without complete documentation of the prescribed tablets (e.g., whether TDF/FTC were given separately or combined) were further excluded from the sub-analysis in which we tested the effect of the pill burden on the treatment success.

Study outcomes

Two primary outcomes were analyzed: virological failure (VF) and emergence of NRTI drug resistance. The latter was defined as the first detection of any major IAS-USA drug resistance mutation [287] to NRTI following VF. VF was defined as HIV-1 plasma RNA level ≥ 400 copies/$m\ell$ after 180 days of continuous treatment. If the subsequent HIV-RNA was < 400 copies/$m\ell$, it was considered as viral blip. Not all patients experiencing VF had a genotypic resistance test (GRT) performed following VF. Including the subjects in the resistance analysis, for whom we could not determine whether resistance emerged or not, would be potentially incorrect because they would be included as if they did not have any resistance. Thus, we first compared the characteristics of those with and without GRT following VF within the same regimen group by Wilcoxon rank-sum test. Variables tested were treatment length, time from treatment initiation to VF, viral load at VF, and the consecutive viral load at VF. If there was no evidence for a difference we excluded those with VF but without GRT from the resistance analysis in order to avoid selection bias.

Statistical methods

We analyzed data with univariable and multivariable Cox proportional hazard models to estimate Hazard ratios (HRs) with 95% confidence intervals (CIs) and used robust standard errors to account for possible intra-patient correlations because a patient could be included twice: 1) first-line cART and 2) switched to cART regimens while suppressed. Exposure time started at treatment initiation for every treatment episode. Patients were censored at the time of death, the last visit date, or the end of the treatment, whichever came first. Regimens were included categorically in the model.
Adjustment comprised all variables with univariable significance, which included age (continuous), ethnicity (white and non-white, categorical), and treatment starting year (continuous), and variables decided a priori including baseline HIV-RNA (log_{10} transformed, continuous) and baseline CD4 counts (square-root transformed, continuous). Baseline CD4 and HIV-RNA data at the initiation of the first cART were retrieved. Missing baseline CD4 counts (5%) and HIV-RNA (7%) were imputed using multivariable normal regression (an iterative Markov chain Monte Carlo method) and estimated by age, sex, ethnicity, inclusion center, transmission route, and treatment starting year. In the pill burden analysis models were adjusted for two more co-variables: pill burden (i.e. the total pill number per day, continuous), and the maximal dosing frequency per day (once or twice-daily); both were time-updated variables. Since CD4 counts or pill burden entered categorically did not improve the model fit, we chose them to be continuous. Collinearity was tested with variance inflation factors and correlation matrices and none was found.
To test the robustness of our results we performed two sensitivity analyses in which we either restricted NNRTI drugs to EFV or restricted our study population to patients on first-line cART. The analyses were performed using Stata 13.0 SE (StataCorp, Texas, USA).

10.3 Results

Study population

9755 patients have been ART-experienced since 1996 in the SHCS. Among these individuals, 2678 had initiated treatment containing one of the regimens of interest and 1338 had switched from any regimen to one of the regimens of interest. Ninety-nine (7%) patients from the switching group were excluded due to VF or drug resistance identified at the time of switching. This resulted in 3917 treatment episodes from 3398 individuals. Baseline GRT was available for 2477/3398 (73%) patients. 77/3398 (2%) patients had low-level resistance to the prescribed regimen and therefore were excluded. In total, our study comprised of 3833 treatment episodes represented by 3321 individuals (Figure 10.1). Baseline characteristics were summarized in Table 10.1. Overall, statistically significant differences between groups were observed in all characteristics except for ethnicity.

Virological failures

The following crude numbers of virological failures were observed for the different NRTI backbones studied (Figure 10.2 A): TDF/FTC, 1858 treatment episodes with 19 failures (1.0%); ABC/3TC, 387 treatment episodes with 9 failures (2.3%); TDF/3TC, 344 treatment episodes with 11 failures (3.2%); AZT/3TC, 1244 treatment episodes with 45 failures (3.6%).
In the univariable model the lowest failure rate was observed for TDF/FTC (as reference), followed by ABC/3TC (HR, 2.38 [95%CI,1.07 - 5.26], $p = 0.033$), TDF/3TC (HR, 4.04

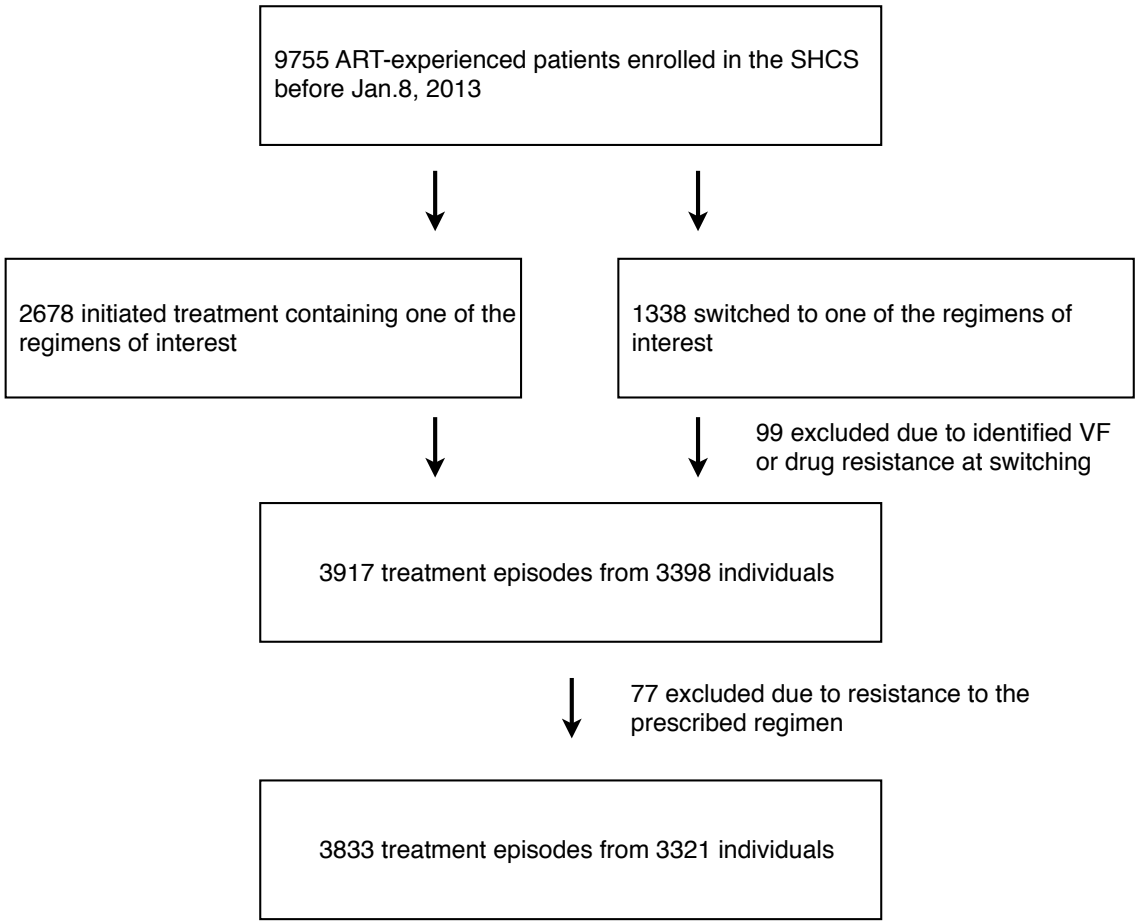


Figure 10.1: Patient selection profile

Treatment group[a]	**TDF/FTC**	**ABC/3TC**	**TDF/3TC**	**AZT/3TC**
	N, (%)	N, (%)	N, (%)	N, (%)
Total number of patients (n=3321)	1577 (47.5)	274 (8.3)	268 (8.1)	1202 (36.2)
Median age at baseline (IQR[b])	40 (32, 46)	40 (33, 48)	39 (32, 45)	37 (31, 44)
Ethnicity				
White	1171 (74.2)	190 (69.3)	204 (76.1)	862 (71.7)
Non-white	406 (25.8)	84 (30.7)	64 (23.9)	340 (28.3)
Gender & Transmission route				
Homosexual Men (MSM)	873 (55.4)	114 (41.6)	94 (35.1)	387 (32.2)
Heterosexual Men (HSXM)	314 (19.9)	67 (24.5)	63 (23.5)	285 (23.7)
Heterosexual Women (HSXF)	252 (16.0)	60 (21.9)	73 (27.2)	310 (25.8)
Intravenous drug users (IDU)	69 (4.4)	18 (6.6)	29 (10.8)	157 (13.1)
Unknown	69 (4.4)	15 (5.5)	9 (3.4)	63 (5.2)
Median CD4 count at baseline (cells/mm^3) (IQR)	282 (190, 383)	250 (173, 343)	200 (104, 287)	208 (122, 313)
Median viral load at baseline ($\log_{10}$ copies/ml) (IQR)	4.5 (3.5, 5.0)	4.2 (2.6, 4.9)	4.7 (3.6, 5.3)	4.7 (3.9, 5.3)
Total number of treatment episodes (n=3833)	1858 (48.5)	387 (10.1)	344 (9.0)	1244 (32.5)
Median treatment initiation year (IQR)	2009 (2008, 2011)	2008 (2007, 2010)	2005 (2004, 2006)	2002 (2000, 2004)
First-line treatment[c]	1292 (69.5)	198 (51.3)	211 (61.3)	912 (73.4)

a. TDF: tenofovir, FTC: emtricitabine, ABC: abacavir, 3TC: lamivudine, AZT: zidovudine
b. IQR: interquartile range
c. percentage in the bracket indicated the ratio to the total number of that specific regimen

Table 10.1: Basic characteristics of study population

	Virological Failure						Virological Failure in pill burden analysis					
	Univariable			Multivariable			Univariable			Multivariable		
	HR	95% CI	P	HR	95 % CI	P	HR	95 % CI	P	HR	95 % CI	P
Regimen[a]												
TDF/FTC	1			1				1		1		
ABC/3TC	2.38	1.07-5.26	0.033	2.01	0.89-4.55	0.095	2.15	0.94-4.91	0.069	1.79	0.76-4.23	0.18
TDF/3TC	4.04	1.92-8.48	<0.001	2.89	1.21-6.88	0.016	4.29	2.04-9.05	<0.001	2.64	0.94-7.44	0.066
AZT/3TC	3.89	2.26-6.69	<0.001	2.28	1.01-5.14	0.046	2.89	1.48-5.63	0.002	3.10	0.51-18.7	0.22
Age, increase per year	0.97	0.95-0.99	0.005	0.99	0.96-1.01	0.23	0.98	0.96-1.01	0.22	1.00	0.97-1.03	0.95
Ethnicity												
White	1			1			1			1		
Non-white	2.94	1.91-4.53	<0.001	2.67	1.69-4.23	<0.001	2.90	1.70-4.97	<0.001	2.85	1.64-4.96	<0.001
Gender & Transmission route[b]												
MSM	1			-			1			-		
HSXM	2.23	1.28-3.88	0.005	-			2.21	1.16-4.19	0.015	-		
HSXF	1.84	1.00-3.38	0.051	-			1.61	0.76-3.40	0.22	-		
IDU	2.24	1.04-4.83	0.040	-			1.24	0.37-4.21	0.73	-		
Unknown	2.72	1.11-6.68	0.029	-			2.06	0.61-7.02	0.25	-		
Square-root of CD4 count	0.95	0.91-0.99	0.023	0.97	0.92-1.02	0.30	0.95	0.90-0.99	0.03	0.97	0.92-1.03	0.35
Log_{10} viral load	1.14	0.95-1.36	0.17	1.07	0.88-1.30	0.50	1.18	0.95-1.46	0.13	1.13	0.89-1.44	0.31
Treatment starting year, increase per year	0.87	0.82-0.92	<0.001	0.94	0.85-1.04	0.22	0.90	0.83-0.97	0.005	1.05	0.93-1.19	0.40
Pill number per day	-			-			1.69	1.31-2.19	<0.001	1.41	1.01-1.96	0.043
Dosing frequency per day	-			-			1.81	1.02-3.19	0.041	0.64	0.12-3.30	0.59

a. TDF: tenofovir, FTC: emtricitabine, ABC: abacavir, 3TC: lamivudine, AZT: zidovudine
b. MSM: men having sex with men, HSXM: heterosexual men, HSXF: heterosexual women, IDU: intravenous drug users

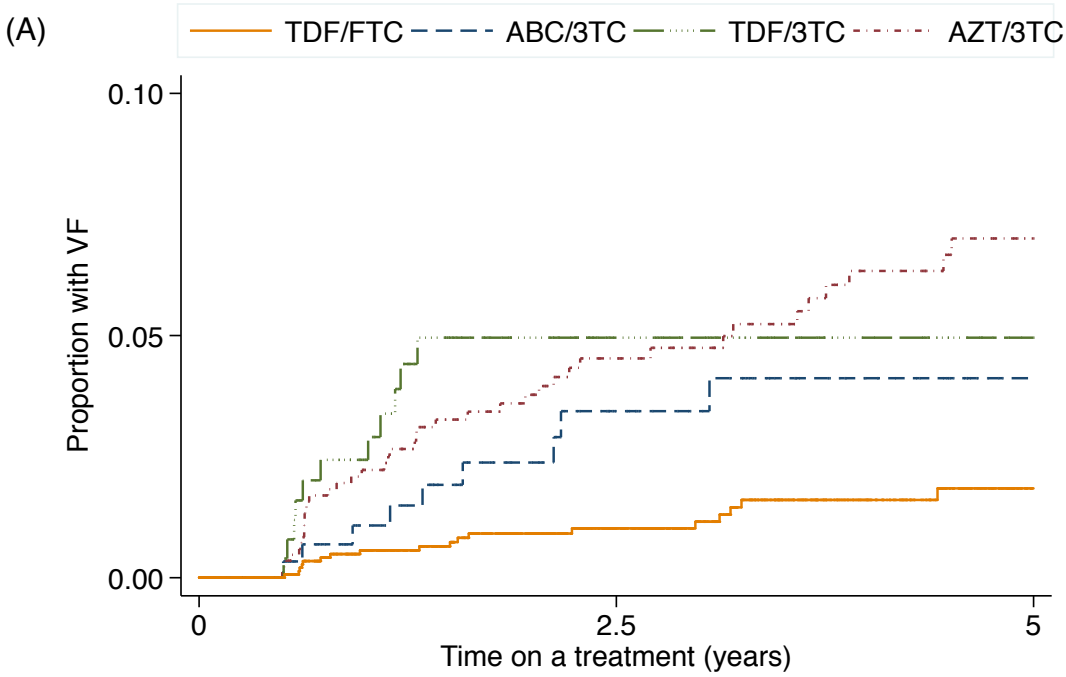
Table 10.2: Uni- and multivariable cox proportional hazard analysis for virological failure

The multivariable models were adjusted for all variables indicated, i.e., showing HR

[1.92 - 8.48], $p < 0.001$) and AZT/3TC (HR, 3.89 [2.26 - 6.69], $p < 0.001$; Table 10.2, left part). After adjustment for baseline CD4 count, baseline HIV-1 RNA and all significantly associated co-variables including age, ethnicity, and year of treatment start, HRs among regimens decreased in magnitude (ABC/3TC: 2.01 [0.89 - 4.55], $p = 0.095$; TDF/3TC: 2.89 [1.22 - 6.88], $p = 0.016$; AZT/3TC: 2.28 [1.01 - 5.14], $p = 0.046$). Ethnicity was strongly associated with treatment outcome: the non-white patients were 2.67 times more likely to experience VF than the white population (95% CI, 1.69 - 4.23, $p < 0.001$).

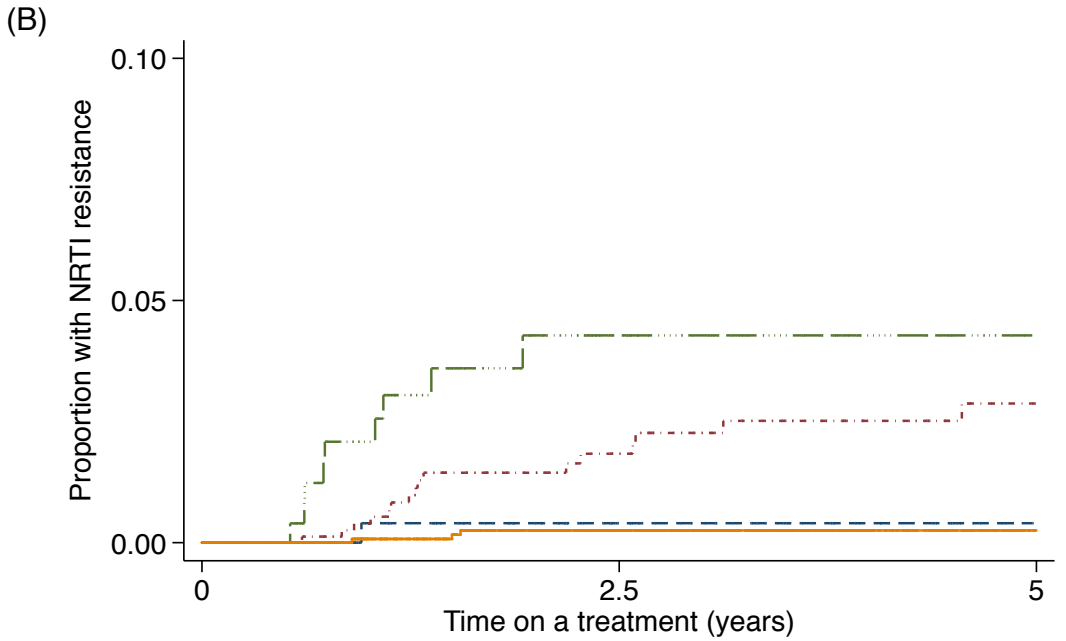
Emergence of NRTI resistance

Next we analyzed the relative efficacy of the four NRTI combinations regarding time to the emergence of any NRTI resistance mutation following VF. However, 9 out of 19 (47%) failing regimens with TDF/FTC were not genotyped, and the numbers of non-genotyped treatments from failing treatments containing ABC/3TC, TDF/3TC, and AZT/3TC, respectively were 4 from 9 (44%), 1 from 11 (9%), and 16 from 45 (36%). Wilcoxon rank-sum test did not find an indication for a difference between patients with and without GRT following VF. Because we had no alternative means to determine whether drug resistance had developed in a non-genotyped failure episode, we excluded those without GRT from this analysis. After exclusion, we detected NRTI resistance in 3 of 1849 (0.2%) TDF/FTC, 1 of 383 (0.3%) ABC/3TC, 9 of 343 (2.6%) TDF/3TC, and 17 of 1228 (1.4%) AZT/3TC containing regimens that failed (Figure 10.2 B).
In the univariable model the HR of ABC/3TC showed no evidence for an effect on the emergence of resistance when compared to TDF/FTC (1.72 [0.18 - 16.5], $p = 0.64$), but more emergence of NRTI resistance was associated with TDF/3TC (20.4 [5.49 - 75.6], $p < 0.001$) and AZT/3TC (9.8 [2.88 - 33.3], $p < 0.001$; Table 10.3, left part). The



No. at risk

TDF/FTC	1858	(13)	859	(5)	319
ABC/3TC	387	(8)	161	(1)	78
TDF/3TC	344	(11)	97	(0)	17
AZT/3TC	1244	(32)	467	(9)	244



No. at risk

TDF/FTC	1849	(3)	856	(0)	319
ABC/3TC	383	(1)	161	(0)	78
TDF/3TC	343	(9)	98	(0)	17
AZT/3TC	1228	(12)	467	(4)	243

Figure 10.2: Kaplan-Meier curves for time to (A) VF and (B) emergence of NRTI resistance in the different treatment groups

TDF indicates tenofovir; FTC, emtricitabine; ABC abacavir; 3TC, lamivudine; AZT, Zidovudine. The numbers of failure events are shown in parentheses.

	NRTI Resistance						NRTI Resistance in pill burden analysis					
	Univariable			Multivariable			Univariable			Multivariable		
	HR	95% CI	P	HR	95 % CI	P	HR	95 % CI	P	HR	95 % CI	P
Regimen[a]												
TDF/FTC	1			1			1			1		
ABC/3TC	1.72	0.18-16.5	0.64	1.17	0.11-12.2	0.90	1.74	0.18-16.7	0.63	1.16	0.10-12.9	0.91
TDF/3TC	20.4	5.49-75.6	<0.001	11.3	2.34-55.3	0.003	21.0	5.63-78.0	<0.001	5.60	0.71-44.0	0.10
AZT/3TC	9.80	2.88-33.3	<0.001	4.02	0.78-20.7	0.096	5.84	1.42-24.1	0.015	1.62	0.19-14.1	0.66
Age, increase per year	0.94	0.91-0.98	0.002	0.97	0.94-1.01	0.21	0.96	0.92-1.00	0.058	0.98	0.94-1.03	0.45
Ethnicity												
White	1			1			1			1		
Non-white	5.41	2.55-11.5	<0.001	4.43	1.85-10.6	0.001	3.84	1.51-9.78	0.005	3.54	1.20-10.4	0.022
Gender & Transmission route[b]												
MSM	1			-			1			1		
HSXM	2.85	1.15-7.10	0.024	-			2.98	0.95-9.36	0.061	-		
HSXF	2.48	0.93-6.58	0.068	-			2.64	0.76-9.11	0.13	-		
IDU	0.76	0.09-6.11	0.80	-			-	-	-	-		
Unknown	2.76	0.58-13.0	0.20	-			2.43	0.28-21.0	0.42	-		
Square-root of CD4 count	0.88	0.83-0.94	<0.001	0.88	0.81-0.96	0.002	0.88	0.81-0.94	<0.001	0.89	0.80-1.00	0.042
Log_{10} viral load	1.03	0.73-1.45	0.86	0.87	0.67-1.15	0.33	1.30	0.78-2.19	0.32	1.05	0.60-1.83	0.86
Treatment starting year, increase per year	0.81	0.75-0.87	<0.001	0.91	0.78-1.06	0.23	0.79	0.73-0.86	<0.001	0.98	0.78-1.24	0.90
Pill number per day	-			-			2.83	1.95-4.10	<0.001	1.72	0.97-3.02	0.062
Dosing frequency per day	-			-			2.33	0.91-5.99	0.078	1.19	0.24-5.82	0.83

a. TDF: tenofovir, FTC: emtricitabine, ABC: abacavir, 3TC: lamivudine, AZT: zidovudine
b. MSM: men having sex with men, HSXM: heterosexual men, HSXF: heterosexual women, IDU: intravenous drug users

Table 10.3: Uni- and multivariable cox proportional hazard analysis for emergence of NRTI resistance

The multivariable models were adjusted for all variables indicated, i.e., showing HR

adjusted HR on emergence of NRTI resistance for patients on TDF/3TC compared to TDF/FTC was 11.3 (2.34 - 55.3, $p = 0.003$), but HR was not significantly different for ABC/3TC (1.17 [0.11 - 12.2], $p = 0.90$) or AZT/3TC (4.02 [0.78 - 20.7], $p = 0.096$). However, ethnicity was strongly associated with the emergence of NRTI resistance (non-white versus white: HR, 4.43 [1.85 -10.6], $p = 0.001$) in the adjusted model.

Study population in the pill burden analysis

Since pill number and dosing frequency were essential for the pill burden analysis, patients without full documentation of tablet usage were excluded from this analysis, resulting in 3088 treatment episodes from 2684 individuals. Given that pill burden and dosing frequency are time-updated variables, we had 4263 observations in total. Median, minimal and maximal numbers of total pills were 2, 1 and 6. For the TDF/FTC, ABC/3TC, TDF/3TC, and AZT/3TC regimen groups the median (IQR) number of total pills was 2 (1,2), 2 (2,3), 4 (4,4), and 3 (3,3), respectively, and the proportions of once-daily regimens were 97.2%, 92.5%, 81.0%, and 1.4%.

Virological failures in the pill burden analysis

Similar to the original analysis, the univariable analysis of pill burden resulted in the lowest chance of having VF from TDF/FTC group when compared to the other three regimens (ABC/3TC: HR, 2.15 [0.94 - 4.91], $p = 0.069$; TDF/3TC: 4.29 [2.04 - 9.05], $p < 0.001$; AZT/3TC: 2.89 [1.48 - 5.63], $p = 0.002$; Table 10.2, right part). However, contrary to the original analysis, in which pill burden and dosing frequency were not adjusted, the

multivariable model did not show evidence for regimen differences (ABC/3TC: HR, 1.79 [0.76 - 4.23], $p = 0.18$; TDF/3TC: 2.64 [0.93 - 7.44], $p = 0.066$; AZT/3TC: 3.10 [0.51 - 18.7], $p = 0.22$). Pill burden was associated with VF both in the univariable (HR,1.69 [1.31 - 2.19], $p < 0.001$; Figure 10.3 A) and the multivariable models (HR,1.41 [1.01 - 1.96], $p = 0.043$). In addition, ethnicity (HR,2.85 [1.64 - 4.96], $p < 0.001$) remained a strong predictor for having VF after adjustment. In contrast to pill burden, we did not find association of dosing frequency with VF after adjustment (HR, 0.64 [0.12 - 3.30], $p = 0.59$).

Emergence of NRTI resistance in the pill burden analysis

Results followed the same pattern as for results on VF in the pill burden analysis. Evidence for effects of TDF/3TC and AZT/3TC in the univariable model (ABC/3TC: HR, 1.74 [0.18 - 16.7], $p = 0.63$; TDF/3TC: 21.0 [5.63 - 78.0], $p < 0.001$; AZT/3TC: 5.84 [1.42 - 24.1], $p = 0.015$; Table 10.3, right part) was not found after adjustment (ABC/3TC: HR, 1.16 [0.10 - 12.9], $p = 0.91$; TDF/3TC: 5.60 [0.71 - 44.0], $p = 0.10$; AZT/3TC: 1.62 [0.19 - 14.1], $p = 0.66$), but ethnicity remained a strong risk factor (HR,3.54 [1.20 - 10.4], $p = 0.022$). At the same time, we observed a significant effect of pill burden in the univariable model (HR, 2.83 [1.95 - 4.10], $p < 0.001$; Figure 10.3 B) and a trend in the multivariable model (HR, 1.72 [0.97 - 3.02], $p = 0.062$) to be associated with the emergence of NRTI resistance. Dosing frequency, however, again showed no effect on the emergence of NRTI resistance (HR, 1.19 [0.24 - 5.82], $p = 0.83$).

Sensitivity analyses

Sensitivity analyses, in which we restricted NNRTI to EFV or our study population to patients on first-line treatment only, robustly showed qualitatively similar results. When adjusted additionally for pill burden and dosing frequency, evidence for effects of regimens both on VF and on the emergence of NRTI resistance was not detected. However, one distinct exception was observed: the ABC/3TC treatment group became a stronger predictor for VF in the model restricting to the first-line patients (HR, 2.93 [1.06 - 8.13], $p = 0.039$; pill burden analysis: HR, 3.13 [1.10 - 8.87], $p = 0.032$). Pill burden was constantly observed to have an impact (restricting to first-line on VF: HR, 1.46 [1.01 - 2.13], $p = 0.046$; on resistance: HR, 2.25 [1.21 - 4.18], $p = 0.011$; restricting to EFV on VF: HR, 1.66 [1.17 - 2.37], $p = 0.005$; on resistance: HR, 2.14 [1.04 - 4.41], $p = 0.038$) and ethnicity remained strongly predictive of experiencing VF and emergence of NRTI resistance when restricting to first-line (on VF: HR, 2.66 [1.56 - 4.51], $p < 0.001$; on resistance: HR, 3.32 [1.36 - 8.15], $p = 0.009$; in pill burden analysis on VF: HR, 2.71 [1.41 - 5.21], $p = 0.003$; on resistance: HR, 2.50 [0.76 - 8.28], $p = 0.13$) and restricting to EFV (on VF: HR, 2.74 [1.67 - 4.47], $p < 0.001$; on resistance: HR, 5.52 [2.01 - 15.2], $p = 0.001$; in pill burden analysis on VF: HR, 3.17 [1.72 - 5.85], $p < 0.001$; on resistance: HR, 4.89 [1.25 - 19.2], $p = 0.023$).

10.4 Discussion

In this large study, representative of the treatment history of a whole country, we compared virological failure rates and emergence of NRTI resistance of four major NRTI combinations TDF/FTC, ABC/3TC, TDF/3TC, and AZT/3TC combined with either EFV or NVP in a real-world clinical setting. Treatment failure rates and frequency of resistance emergence was remarkably low in our setting. Failure frequencies ranged from 1.0% for the TDF/FTC to 3.6% for the AZT/3TC treatment group, and the rate of NRTI resistance

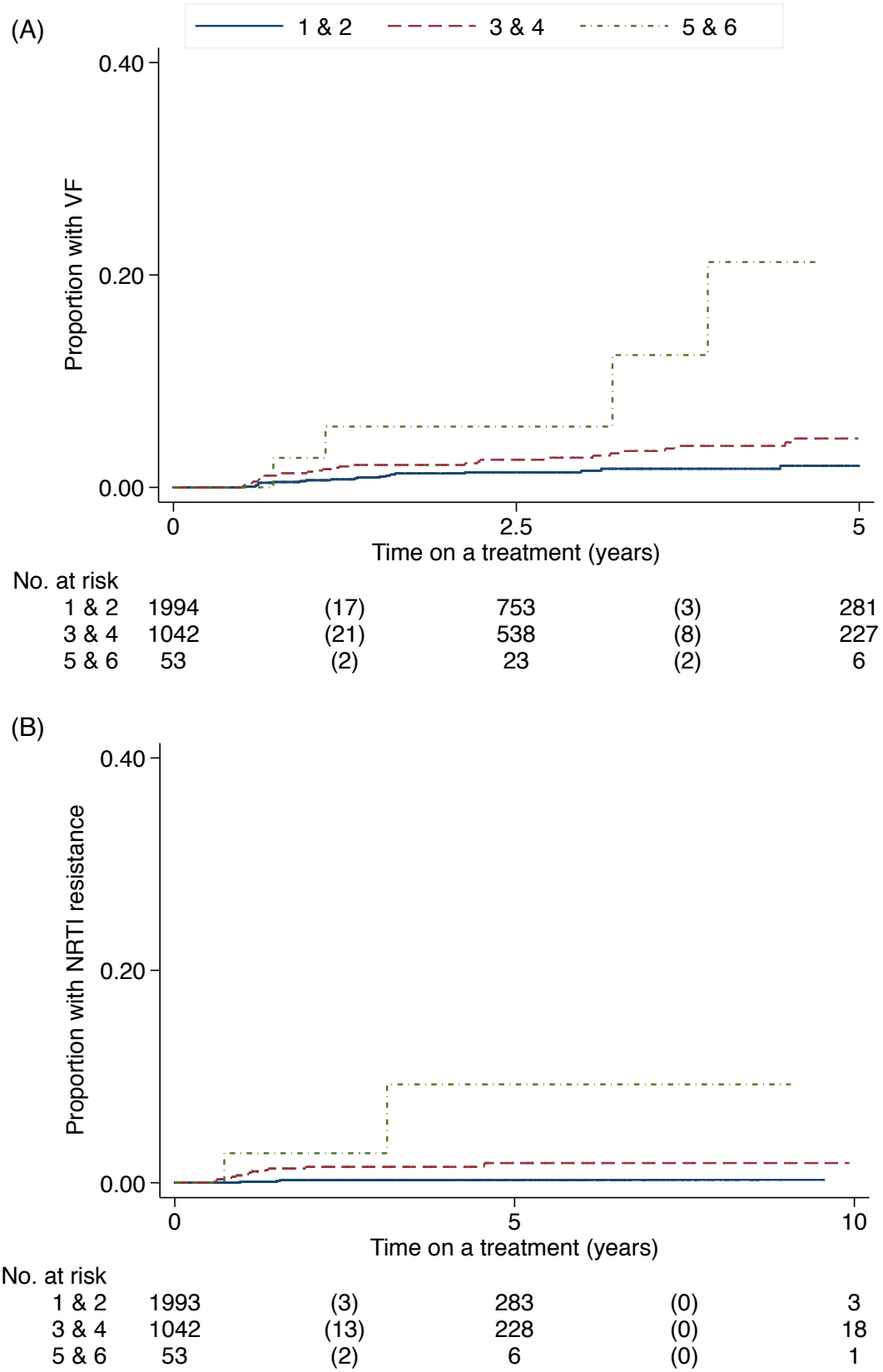


Figure 10.3: Kaplan-Meier curves for time to (A) VF and (B) emergence of NRTI resistance in the different treatment groups

TDF indicates Tenofovir; FTC, Emtricitabine; ABC Abacavir; 3TC, Lamivudine; AZT, Zidovudine. The numbers of failure events are shown in parentheses.

was even lower ranging from 0.2% for TDF/FTC to 2.6% for TDF/3TC. In univariable and multivariable analyses we found that ABC/3TC, TDF/3TC and AZT/3TC containing regimens had a more than two folds higher risk leading to VF than TDF/FTC containing regimens. TDF/3TC was more often associated with emergence of NRTI-resistance than TDF/FTC. Among regimens other than TDF/FTC, no clear superiority was found. When adjusting for pill burden and dosing frequency, we found that higher number of pills but not dosing frequency was associated with VF and emergence of NRTI drug resistance. Additionally, a very strong predictor of VF and resistance emergence across all analyses was ethnicity. Specifically, treatment responses in white patients compared to non-white patients were better.

Our findings are of high relevance in the light of the upcoming availability of generic drugs. It can be envisioned that health care systems and health insurance companies may build up considerable pressure on health care providers to use cheap drugs independent of pill numbers, tolerability or toxicity. Here, we showed that this strategy most likely would result in higher rates of treatment failures and more frequent emergence of resistance. Thus, even when generics are available, the aim to minimize the pill burden should be maintained, however, dosing frequency is less important in this regard.

The strength of this study was the representativeness. On the other hand, limitations of our study were that the sample size was not large enough to differentiate more between NRTI backbones and that events of VF or NRTI resistance were few. However even in the pill burden analysis in which we did not find evidence for an effect between regimens, HR of ABC/3TC, TDF/3TC, and AZT/3TC comparing to TDF/FTC for having VF and emergence of NRTI resistance were indeed all above one but had very wide CI. To this point it was difficult to determine whether power issues have limited our ability to document evidence. Studies with more individuals are needed to evaluate the relative efficacy of the regimens and to disentangle the effects of pill burden and the type of regimen. However, even with our sample size we could observe effects of pill burden and ethnicity. Hence, our data suggested that both ethnicity and pill burden was at least equally important as treatment itself in affecting treatment efficacy and had decisive impact on VF and the emergence of NRTI resistance.

The effect on viral responses of pill burden but not dosing frequency found in our analysis was consistent with results from a recent meta-analysis [151]. A large and long-term randomized trial also found that twice-daily regimens containing Raltegravir performed at least as well as once-daily regimens [288]. Additionally, in a previous study, sub-Saharan African patients in Switzerland showed a higher risk of virological failure on cART [289]. Their inferior self-reported adherence [289] [148] could possibly explain the strong and robust effect of ethnicity found in our analyses.

Our study included not only treatment-naïve patients initiating their first cART but also patients switching from the first cART if they did not have VF or resistance detected at or before switching. Several potential problems should be noted in this respect: First, second-line patients started treatment with fully suppressed HIV-1-RNA. As a result, the risk of developing drug resistance could be smaller than for first-line patients because failure during the time to achieve viral suppression was not possible by definition. However, this was the case for all four regimens, thus no bias could exist. Second, for patients whose NRTI backbone was identical in the first and the second cART (i.e., only the third agent was changed), it was possible that drug resistance had developed as minor variants during the first cART but was not detected. As a result, second-line treatment might have failed sooner than first-line treatment. However, sensitivity analyses including only first-line patients confirmed that our results were robust, apart from the higher HR of ABC/3TC containing regimens on VF. The difference of ABC/3TC suggested again that

studies with a larger sample size are needed in determining the relative efficacy of these NRTI combinations.

In conclusion, pill burden and ethnicity showed robust associations with virological responses and emergence of NRTI resistance. Studies with more individuals and failure events are needed to disentangle the effects of pill burden and the drug regimens.

11

Papers with co-authorship

Personal Contributions

During my thesis I selected samples for sequencing for my thesis projects, which are also of high importance for the following projects and publications:

1. Treatment-Naive Individuals Are the Major Source of Transmitted HIV-1 Drug Resistance in Men Who Have Sex With Men in the Swiss HIV Cohort Study
   Sara M. Drescher, Viktor von Wyl, Wan-Lin Yang, Jürg Böni, Sabine Yerly et al.
   Clin Infect Dis. 2014 Jan;58(2):285-94

2. Higher Risk of Incident Hepatitis C Virus Coinfection Among Men Who Have Sex With Men, in Whom the HIV Genetic Bottleneck at Transmission Was Wide
   Roger D. Kouyos, Andri Rauch, Dominique L. Braun, Wan-Lin Yang, Jürg Böni et al.
   JID 2014 Oct 24; 210(10):155561

3. Estimating the dynamics and dependencies of accumulating mutations
   Hesam Montazeri, Huldrych F. Günthard, Wan-Lin Yang, Roger D. Kouyos, Niko Beerenwinkel
   In revision in Biostatistics (BIOSTS-14228)

III

Discussion and References

12 Discussion

Drug resistance is one of the major concerns jeopardizing treatment success for bacterial, mycobacterial, parasitic, and viral infections. It is almost inevitable that, whenever antimicrobial treatments become available, drug resistance to those treatments emerges over time. This also holds true for HIV-1. Despite the great success of antiretroviral treatment, drug resistance remains an obstacle to control HIV-1 replication at both the individual and population levels. Although there are more than 25 antiretroviral drugs available today and new drugs are continuously developed, treatment options are still limited for some HIV-1 infected individuals mostly due to drug resistance acquired from suboptimal therapies (e.g., mono- or dual-therapy), with which they were treated in the early days. Fortunately, since very early on, the newest technologies and high quality surveillance methods have been applied to the HIV pandemic. Longitudinal observational cohorts enabled the study of the emergence and mechanisms of resistance from the very beginning in a systematic way for this disease.

For every observational epidemiological study, a representative database is essential for achieving high quality. The SHCS and the SHCS drug resistance databases are characterized by a high quality and a high density of data. They provide a highly valuable resource for many epidemiologists to study the many facets of HIV-1 including drug resistance at the population level.

Acquired HIV-1 drug resistance represents the initial source of circulating resistance mutations. In most resource-rich countries, a decreasing prevalence of acquired drug resistance mutations has been documented over the years; however, the prevalence of transmitted drug resistance mutations has not decreased in these countries. I studied this apparent paradox in my Research Project 1. The aim of this project was to understand the dynamics between acquired and transmitted drug resistance at a population level. This project shows that transmission of HIV-1 drug resistance mutations was under a transient control when a new drug class was introduced, but increased in the years when no new drug was introduced. It also shows that treatment-naïve individuals formed an important source for transmitted drug resistance mutations, especially mutations with lower fitness cost.

It is generally believed that differential persistence of transmitted drug resistance mutations is caused, at least partially, by variations in fitness costs of the mutations. In my Research Project 2 I used *in vivo* data to study this topic, and found that transmitted drug resistance mutations persist longer in the absence of drug pressure when these mutations have a lower fitness cost, which is both true for fitness cost among different mutations and among different genetic backgrounds of the same mutation. As a result, higher fitness mutations, either by nature or by regained fitness through compensatory

mutations, persist longer in a treatment-naïve population, and have a greater chance to be transmitted and to survive the transmission bottleneck.

These results raise a warning: Switzerland has optimized clinical and public health circumstances with universal access to antiretroviral treatment and extensive use of drug resistance and viral load testing. Therefore, even in an optimal setting, it appears infeasible to reduce the transmission of drug resistance mutations. Consequently, containing the HIV-1 epidemic and transmission of HIV-1 drug resistance in resource-limited countries will be more challenging. Limitation of drug options in these countries could lead to increasing prevalence of transmitted drug resistance mutations over time. Moreover, especially mutations in genetic backgrounds that cause lower fitness costs would have a higher chance to spread. In resource-limited settings where surveillance of the HIV-1 infection and drug resistance is usually poor, and availability of different drug classes is limited for large populations, specific mutations may dominate in the infected populations in the long run. Improving surveillance of the HIV-1 infection, enhancing availability of all drug classes universally, and treating infected patients early on play an important role in containing transmission of drug resistance and transmission of HIV in general.

In addition to containing the spread of transmitted drug resistance, another key factor to successfully reduce the number of HIV-1 infection is the availability of potent treatment. Newer drugs have been found to be less toxic and more potent. The issue of reducing pill burden has also gained increasing attention. In Research Project 3, I took into account the possible influence of pill burden and dosing frequency on treatment efficacy, and compared the relative potency of the four mostly used NRTI combinations over time: TDF/FTC, ABC/3TC, TDF/3TC, and AZT/3TC. These four combinations range from more historical NRTI drugs, which have more tolerability issues, higher dosing frequency, and higher pill burden, to the latest NRTI drugs, which have the option of the once-daily one-pill formula. Results showed that, in addition to the effect of regimens themselves, higher pill burden and non-white patients are more associated with experiencing virological failures and the emergence of NRTI resistance. Therefore, pill burden and ethnicity might play an important role in influencing treatment efficacy.

Drug resistance is a complex and dynamic topic. These three research projects contributed to a better understanding of the interaction of acquired and transmitted drug resistance at a population level. We believe that containing drug resistance is certainly one of the key factors in controlling the HIV-1 infection. It is therefore important to continuously monitor transmission and emergence of drug resistance, and to start treatment early. Continuously developing new drugs and making all drugs universally available are also of high importance. For this purpose, maintaining longitudinal observational databases of high quality in different parts of the world are essential efforts to control the HIV-1 epidemic, in particular, because eradication or a vaccine are not yet within reach.

13 References

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Date	Presentation	Symposium	Title
07.03.12	Poster	CROI 2012	15-year prevalence data of transmitted drug resistance shows a positive association with mean population viral load of treatment-failing patients from the previous year
19.04.12	Poster	Day of Clinical Research, USZ, 2012	15-Year Prevalence Data of HIV-1 Transmitted Drug Resistance in Switzerland
05.06.13	Talk	International Workshop on HIV & Hepatitis Virus Drug Resistance and Curative Strategies 2013	Prevalence of transmitted drug resistance and relation to mean population viral load of treatment failing patients: a 16-year analysis within the Swiss HIV Cohort Study (SHCS)
21.08.13	Talk	Club de Pathologie Infectieuse 2013	Prevalence of transmitted drug resistance and relation to mean population viral load of treatment failing patients: a 16-year analysis within the Swiss HIV Cohort Study (SHCS)
04.03.14	Poster	CROI 2014	Persistence of Transmitted HIV-1 Drug Resistance Mutations Associated with Fitness Costs
05.06.14	Poster	International Workshop on Antiretroviral Drug Resistance 2014	Abacavir-Lamivudine And Tenofovir-Emtricitabine Are Superior NRTI Options for Antiretroviral Treatment to Zidovudine-Lamivudine Or Tenofovir-Lamivudine Paired with NNRTI

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List of Publications

Sara M. Drescher, Viktor von Wyl, **Wan-Lin Yang**, Jürg Böni, Sabine Yerly, Cyril Shah, Vincent Aubert, Thomas Klimkait, Patrick Taffé, Hansjakob Furre, Manuel Battegay, Juan Ambrosioni, Matthias Cavassini, Enos Bernasconi, Pietro L. Vernazza, Bruno Ledergerber, Huldrych F. Günthard, Roger D. Kouyos, and the Swiss HIV Cohort Study. Treatment-Naive Individuals Are the Major Source of Transmitted HIV-1 Drug Resistance in Men Who Have Sex With Men in the Swiss HIV Cohort Study. Clin Infect Dis. 2014 Jan;58(2):285–94.

Roger D. Kouyos, Andri Rauch, Dominique L. Braun, **Wan-Lin Yang**, Jürg Böni, Sabine Yerly, Thomas Klimkait, Vincent Aubert, Cyril Shah, Helen Kovari, Alexandra Calmy, Matthias Cavassini, Manuel Battegay, Pietro L. Vernazza, Enos Bernasconi, Bruno Ledergerber, Huldrych F. Günthard, and the Swiss HIV Cohort Study (SHCS). Higher Risk of Incident Hepatitis C Virus Coinfection Among Men Who Have Sex With Men, in Whom the HIV Genetic Bottleneck at Transmission Was Wide. J Infect Dis. 2014 Oct 24;210(10):1555–61.

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